A STUDY OF THE BACTERIOLOGY OF PERTUSSIS, WITH SPECIAL REFERENCE TO THE AGGLUTINATION OF THE PATIENT'S BLOOD.*

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Investigations on the subject of the etiology of pertussis by means of microscopic and cultural studies of the sputum have resulted in the finding and description of protozoa by Deichler, Kurloff, and Behla, of staphylococci by Moncorvo and Silva Aronja, of streptococci by Mircoli, of diplococci by Ritter and his pupil Buttermilch, and of bacilli. The last may be divided into three groups: First, a motile bacillus growing "colon-like" upon all ordinary media and forming endogenous spores, described by Affanassieff in 1887, and found later by Szemtschenko and by Wendt. Second, the bacillus found by Czeplewski and Hensel, and also by Koplik, in 1897, growing as a small, poled bacillus upon agar, Löffler's serum, gelatine, and in broth. Zusch, Cavasse, and Arnheim all confirmed Czeplewski's work. The third group comprises the influenza-like bacilli described by Spengler in 1897 and by Jochmann and Krause in 1901 and again in 1903, when they reported the bacilli present in sixty samples of pertussis sputum and in the lungs of twenty-three pertussis patients who died of broncho-pneumonia. They called the organism *Bacillus pertussis*, Eppendorf. It was not found in cases of ordinary broncho-pneumonia, nor in the sputum from other than pertussis cases. Spengler later gave it as his opinion that the description of the sputum smears by Czeplewski and Hensel showed that these observers had been dealing with the same organisms as himself, but that, having failed to use hæmoglobin agar, the influenza-like organisms were lost and only a contaminating pseudo-diphtheria bacillus grew. The fact that

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Czeplewski and Hensel described their bacillus as negative to Gram's stain in sputum, but positive in culture, lends weight to this view. Vincenzi's small bacillus, negative to Gram's stain, grew on all media. Elmassian (1899) found a bacillus which was morphologically like the influenza bacillus, but grew on agar containing human serum as well as on hæmoglobin agar. This organism he found in eight of twenty-two cases of pertussis, but also in cases of tuberculosis and bronchitis in infants and adults, and to it he ascribes no specifie importance. Luzzatto found two organisms: one he identified as Diplococcus lanceolatus, the other as a bacillus closely resembling Bacillus influenzæ, differing from it by the superficial granulation of its colonies and the fact that it grew on serum agar as well as on hæmoglobin agar.

Late in 1903 Manicatide published the results of the examination of eighty cases of pertussis in which agar plates revealed the presence of "bacillus z" in sixty-seven. Horses and sheep were inoculated with broth cultures, and with the serum of these animals cases of pertussis were successfully treated. He is the only investigator who records agglutination tests with the patients' blood, and he records only five cases which gave a definite microscopic reaction in dilutions of 1:32.

At Dr. Flexner's suggestion, working as a Fellow of the Rockefeller Institute for Medical Research, I began a study of the sputum of the children in the pertussis ward of the New York Foundling Asylum, with the special purpose of testing the possible agglutination reactions of the children's blood with the bacilli isolated. The specimens were obtained as follows: at the end of a paroxysm of coughing the sputum was caught in a sterile, covered dish and taken at once to the laboratory; the most solid particle was washed in six successive beakers of peptone water, according to the method of Koch and Kitasato, and finally was spread on blood-agar plates made by mixing placental blood with melted agar.

Smears made from the sputum were stained by Gram's method and with dilute carbol-fuchsin as recommended by Czeplewski and Hensel.

The sputum smears were much alike, showing cocci in pairs,

groups, and, most frequently, in chains, but all comparatively less numerous than bacilli, which were present in every case. These were short, plump, ovoid, with rounded ends, lying singly or in small groups or heaps between pus and epithelial cells; a few were also found within the cells. They were negative to Gram's stain. In some of the cases a larger, slightly curved, Gram positive bacillus was also present. There was a marked contrast between the smears made from the washed and those from the unwashed sputum. In the latter, cocci and Gram positive bacilli predominated, the small bacilli being found but sparingly. In the washed sputum, on the other hand, the small Gram negative bacilli were very numerous, diplococci and long chains of streptococci being present in a few cases. Thus it would appear that these bacilli were not a mouth contamination, but present in the secretion of the bronchi.

On the blood-agar plates the predominating colonies were small, transparent, dewdrop-like, and not surrounded by a hæmolytic zone like that found around the pneumococcus and staphylococcus colonies. Under the microscope the colonies appear as slightly raised drops, almost structureless. In forty-eight hours they have acquired a slightly granular centre; the periphery is quite regular. They do not increase in size after twenty-four hours. On blood-agar slants these bacilli grew as a delicate, raised, moist growth in which the separate colonies can be distinguished until the medium is quite dried up. On ascitic-fluid agar, glycerine agar, Löffler's serum, bouillon, serum broth, milk, and gelatine no growth takes place. In bouillon to which a drop of fresh or hæmolyzed blood has been added, they grow well, but not profusely; an alkaline reaction favored their growth. The bacilli are not motile. They bear transplantation for from eight to ten weeks, but die as soon as the medium becomes dry. From five to twenty-seven days were the limits of life between transplantation, but as a rule the cultures were not alive after eight days. In young cultures the bacilli are all plump, and of about the same size. In older cultures (from three to five days) some larger elements appear; and at first many tubes were discarded as contaminated, until, on testing with serum-agar

tubes and fresh blood-agar, it became evident that the larger bacilli were involution forms, the "Scheinfaden" described by Spengler and by Jochmann, with whose Bacillus Eppendorf this organism seems to be identical. The very long chains which Spengler mentions I have never seen. These bacilli were found in twenty-nine of the thirty cases examined. In the negative case it was not possible to obtain a second specimen.

Other colonies on the plates were identified as pneumococci, streptococci in very long chains, Micrococcus catarrhal is(?), and pseudo-diphtheria bacilli.

Agglutination tests were made with all the varieties of bacilli isolated from each case. In no instance did the Gram positive bacillus give any reaction with the serum of the child from which it was isolated. The influenza-like organisms reacted with the serum of the child in dilutions of 1:200, and occasionally in 1:500. The tests were made according to the technique recommended by Cantani, macroscopically in salt-water suspensions, and controlled by the microscope. The clumps are small, but no free bacilli remain outside them, and the fluid in the small test-tubes becomes more or less clear in from sixteen to twenty hours. Suspensions in distilled water were more successful than in salt solution, as no spontaneous clumping occurs in them. The culture from which the suspension is made should not be more than twenty-four hours old, as older cultures tend to form groups, hard to distinguish from clumps. The reactions were always highest and most complete with homologous serum, but the various strains of the bacillus all reacted with every pertussis blood examined in dilutions of 1:100 at least. The blood from normal cases was tested for control as follows: of three adults, one of whom had never had pertussis, one gave a positive reaction in dilution of 1:10 and the other two were negative; of seven normal children who had never had pertussis, two gave a reaction in a dilution of 1:10 and the other five were negative; of five children who had had pertussis, one and a half to three years before, all gave negative reactions in dilutions higher than 1:10. Swabs from the throats of these twelve children (they did not cough) were all negative for these bacilli.

The blood serum of ten pertussis patients was tested with the influenza bacillus obtained from Dr. Goodwin at the Laboratory of the Board of Health and gave only negative reactions in dilutions higher than 1:20.

In three cases from which the influenza bacillus was isolated from the sputum, the blood gave a positive reaction with the Bacillus influenzæ in dilution of 1:100, but reacted with the bacillus isolated from a pertussis patient in 1:20 only.

Rabbits were immunized with intraperitoneal and subcutaneous injections of twenty-four-hour-old cultures. Before inoculation the blood of the animals gave a fair reaction in a dilution of 1:10, none in 1:20. The agglutinins developed slowly, being positive in 1:500 in eight weeks. The animals lost weight and became emaciated. During the last two weeks of life their agglutinins did not increase in spite of the injection of large doses of bacilli.

The following table shows the reactions of this serum to the influenza and pertussis organisms:

Rabbit I.—Immunized with bacillus from sputum of pertussis case:

	Before Immunization.	After Immunization.
A. Pertussis bacillus	1:10	1:500
	+	+
B. Influenza bacillus	1:10	1:200
	−	+

Rabbit II.—Immunized with bacillus of influenza:

A. Influenza bacillus	1:10	1:200
	+	+
B. Pertussis bacillus	1:10	1:50
	+	+

Absorption tests with these two immunized sera gave the following results:

I. Pertussis immune serum (Rabbit).

A. Saturated with pertussis culture:

	Before Saturation.	After Saturation.
Agglutination of pertussis culture	500	20
Agglutination of influenza culture	200	60

B. Saturated with influenza culture:

Agglutination of pertussis culture	500	100
Agglutination of influenza culture	200	10

	Before Saturation.	After Saturation.
II. Influenza immune serum (Rabbit).		
A. Saturated with influenza culture:		
Agglutination of influenza culture.....	200	10
Agglutination of pertussis culture.....	80	10
B. Saturated with pertussis culture:		
Agglutination of influenza culture.....	200	50
Agglutination of pertussis culture.....	80	0

From these results it is clear that the agglutinins developed in rabbit sera by the inoculation of these two varieties of bacilli are chiefly common to both, and that the so-called specific agglutinins are low in amount, though definitely present. The experiments are being continued in the hope of making the results more positive.

It was noticed throughout the inoculations that the reactions ran fairly parallel in both sera until positive results in dilutions of 1:100 appeared, after which the homologous organism ran quickly ahead of the other.

The bacilli were found with the greatest ease early in the attack, i. e., when the cough had persisted for about two weeks and the whoop was just established. Through the courtesy of Dr. Holt I had an opportunity of examining two cases before the whoop began and failed to find the bacilli, which were very easily isolated at a later examination. The bacilli continued very numerous in the sputum throughout the paroxysmal stage, in several cases even when the attack was eight weeks old. In one case the bacilli were isolated once a week for seven weeks, and the child's blood reacted in a dilution of 1:100 three months after the onset of his cough. Another case reacted eight weeks after he was first seen. Two cases whose serum had given a positive reaction (1:200) during the paroxysmal stage did not react four months after the onset.

As regards animal experiments, white mice, guinea-pigs, and rabbits were inoculated subcutaneously and intraperitoneally without any effect. Dr. Flexner kindly injected a twenty-four-hour culture into the brain of a rabbit, also without any result.

Biologically the bacillus resembles Bacillus influenzæ in its

refusal to grow on any but hæmoglobin-containing media. Morphologically it is slightly larger than the influenza bacillus.

The cases were examined in the months of October, November, and December, at a time when influenza was not prevalent in the city nor in the Foundling Asylum. In January, February, and March pertussis cases were far less numerous than during the preceding months.

Auerbach found influenza bacilli in throat cultures from some of the cases of diphtheria, scarlet-fever, and measles he examined; but the percentage of positive results was always highest during the months when influenza was prevalent in the city (Cologne). He prefers to classify all "influenza-like" bacilli (including the B. pertussis, Eppendorf) as influenza bacilli, and not to assign any specific importance to Jochmann's bacillus. Rosenthal goes still farther, and considers B. influenzæ an ordinary member of the flora of the mouth, and without pathogenic significance. He bases this view upon his findings in cases of broncho-pneumonia at a time when no influenza was present in Paris. The bacillus he isolated grew upon hydrocele agar, but not on horse-serum agar, and Rosenthal considers it identical with the bacillus of Elmassian as well as with the true influenza bacillus. Jochmann and Krause also found, in four pertussis cases, a bacillus similar to that isolated from their other cases, except in its ability to grow on serum as well as on hæmoglobin agar.

I have recently been able to isolate such a bacillus from the nose and throat of a child who was dying of meningitis. The bacillus is morphologically like the influenza and pertussis bacilli, but it grows in fine colonies on ascitic agar, and reacts in the serum of a rabbit immunized to the pertussis organism in dilutions of 1:50 only, the immunizing bacillus reacting in 1:500. This bacillus would seem to be distinct from the true influenza and pertussis bacilli.

The bacilli isolated from this series of pertussis cases belongs to the influenza group, but can be differentiated from Bacillus influenzæ by means of agglutination reactions with the blood of patients and of immunized laboratory animals.

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ON THE PRESENCE OF CERTAIN BODIES IN THE SKIN AND BLISTER FLUID FROM SCARLET-FEVER AND MEASLES.*

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PLATE XXVIII.

INTRODUCTION.

In the *Journal of Medical Research*, Mallory [1] described certain protozoön-like bodies, which he had observed in the epithelial cells and in the lymph spaces of the skin, in material from autopsies on scarlet-fever cases. He was unable to find them in the living patient.

At a meeting of the New York Pathological Society in April, 1904,[2] I reported that I had been able to find these bodies in the skin from five scarlet-fever autopsies, but had been unable to find them in the skin taken from four living patients.

During the summer of 1904, Duval [3] obtained bodies similar to those of Mallory in blister fluid from scarlet-fever patients. In looking over Duval's specimens I was struck by the close resemblance of many of them to the extracellular forms of the malarial parasite, except that in Duval's specimens these bodies showed no chromatin. His specimens were stained with Wright's modification of Leishmann's stain, which, in my experience, does not always give a good chromatin reaction.

Since April, 1904, I have taken skin from twenty scarlet-fever patients, ten scarlet-fever autopsies, fourteen measles patients, four measles post-mortems, four patients with antitoxin rashes, and from five autopsies on diphtheria cases which had had a rash before death. Skin was taken from two children, one of

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whom had died of broncho-pneumonia, the other of marasmus. The skin from each of these cases was divided into four parts and placed in Petri's dishes. One was kept in the ice-box, one at room temperature, one at 37° C., and one at 56° C. While able to obtain many kinds of degeneration products in sections made from these specimens (removed from the Petri's dishes every twenty-four hours under the four conditions), I did not find a picture similar to that shown in the sections from the material taken after death from the cases of scarlet-fever and measles. Though many of the epithelial cells showed masses of varying sizes in their protoplasm, these inclusions showed a difference in staining reaction, some being more acidophilic than the surrounding cytoplasm, others less so, while some are basophilic to a marked extent.

The histological technique followed for all the material was fixation in Zenker's fluid, imbedding in paraffine, and staining with eosin and methylene blue, the sections being, on an average, four microns in thickness.

The bodies found in the material from scarlet-fever and measles were the same, so far as I could determine, as those described by Mallory. Some were intracellular, others lay in the lymph spaces. For the most part they were made up of a delicate reticulum which stained a light blue, the surrounding protoplasm being pink. Only a very few showed the rosettes Mallory described as being so characteristic. In the sections from measles the bodies were not so focal in location as those in scarlet-fever and were found more often in the lymph spaces of the corium; there were also small bodies which showed no reticulum but did show a central nucleus-like granule, these small bodies being found not only in the autopsy material, but also in that from the living patient.

So I can report that in sections made from the skin obtained after death from all of the fifteen cases of scarlet-fever I have been able to find Mallory's bodies. One of these cases was most interesting in that two specimens of skin were obtained, one within five minutes after death and the other twenty-four hours later; in the section made from the former, no bodies could be

made out, but in that from the latter these bodies were easily found. In the twenty-four cases of scarlet-fever, where the skin was taken during life, no bodies were found, except in one section, where it was thought a single small body was seen, but as I have been unable to find it again, this cannot be considered a positive observation. In the material from the four autopsies on cases of measles, cellular inclusions were found in three, one being negative. In the last case the skin was taken one half hour after death, and no other specimen could be obtained. In the material from the fourteen living patients no such bodies as Mallory describes were found, but all showed the small round nucleated bodies. The specimens of skin from the antitoxin rashes were negative, both from the living and the dead patient.

STUDY OF BLISTER FLUID.

Methods.—The method used to obtain blister fluid was that devised by Duval except for a slight modification. A square of adhesive plaster two and one half inches in size was covered with vaseline on its adhesive side, leaving a margin of one half inch. A piece of blotting paper one half inch in diameter and saturated with aqua ammoniæ fortior, was placed in the center of this square, and the whole applied closely to the skin so as to admit no air. After being on from five to seven minutes, the skin was then exposed to the air, when in a short time a blister formed. The fluid was withdrawn from the blister with a sterile capillary tube. Moist spreads were made by blowing a drop of the fluid upon a clean slide and then placing on it a clean cover slip, under which the fluid was thinly and evenly spread. Smears were prepared and were fixed in absolute methyl alcohol for two minutes, some being fixed while dry, others while still moist. I did not find that it made any material difference as to which method was used.

In examining the smears many different stains were used, including a number of the various modifications of the Nocht-Romanowsky stain. In my experience Giemsa's [4] stain was most satisfactory, and Hastings's [5] was almost as good. Hastings's stain was slightly modified; instead of using 100 c. c. of methyl

alcohol to dissolve the dye, I used 50 c. c. of glycerin heated to 60° C., and to this I added the dye, and then 50 c. c. of methyl alcohol which had been previously heated to 60° C. This idea was obtained from Giemsa's method. It permits of a greater concentration of the dye and the glycerin seems to prevent a deposit on the surface of the glass. In using these stains, it is well to over-stain and then decolorize in from fifty to seventy-five per cent. ethyl alcohol or in absolute methyl alcohol which gives a clearer picture.

Blister fluid was taken from eighteen cases of scarlet-fever and from fourteen cases of measles. The bodies of Mallory were found in all the cases of measles and in fourteen out of the eighteen cases of scarlet-fever.

Control Material.—As control material blister fluid was taken from the following cases:

One case of erysipelas.
One case of eczema.
One case of erythema multiforme.
One case of urticaria.
One case of congenital syphilis.
One case of syphilis in the papular stage.
One case of irritated normal skin.
One normal individual.
One case of morbiliform antitoxin rash.
Seven cases of scarlatiniform antitoxin rash.

Material from pustules of two smallpox patients was also examined.

No bodies were found in the blister fluid from any of the above cases except in the last four of the scarlatiniform antitoxin rashes which were studied. In these four cases the blistering fluid was left on the skin for a longer period and caused a more severe irritation. In the material from these cases, bodies were found which it was impossible to differentiate from those found in the blister fluid of measles and scarlet-fever. In one case after withdrawal of the material a moist spread was made and examined at once. Only a very few of the bodies were found, but a number of leucocytes were present. After six hours in the

thermostat the preparation was examined again when many more of these bodies could be demonstrated. On making a smear and staining with Giemsa's solution, these bodies were indistinguishable from those in the blister fluid of cases of measles and scarlet-fever. In these diseases, the bodies are found in the fluid as soon as the rash appears, but not before, and they can be found from four to six days after the appearance of the rash, but as soon as the rash fades away they disappear, becoming fewer and fewer until the sixth day, after which time not one has been observed. If two blisters are applied for from five to seven minutes to one patient, one blister being over a portion of the rash and the other on an area that has no eruption, the bodies can be found in the fluid over the rash; they are also present, though less numerous, in the fluid from the normal skin if the area is blistered for twice as long. The blister fluid from the rashes of both the measles and scarlet-fever patients contained many more leucocytes than that from the other sources.

Conjunctival secretions from twelve cases of measles were examined. In the two cases where bodies similar in appearance to those in the blister fluid were found, there were numerous leucocytes, whereas in the ten negative cases the leucocytes were very few in number.

The Bodies.—In smears of blister fluid stained with Giemsa's solution bodies of various kinds are found. The ones most commonly met with are those having a pale pink body with dark brown or black granules scattered throughout their substance. While many of these are undoubtedly red blood cells, or fragments of protoplasm of degenerating leucocytes, others are coagulated proteid, because similar structures can be found in smears made from horse serum which contained no cellular detritus. The bodies in which most interest centers are those which have the appearance of protozoa, many of them resembling closely the extracellular forms of the malarial parasite. These bodies have a pale blue protoplasm with one or more granules; the granules, which in staining resemble chromatin, vary in size from a mere point to a particle taking up half of the total diameter of the body. Four times these bodies were found with the granules arranged

about the periphery of the cell and with fine lines running to the center of the body, which gave them the appearance of a malarial rosette. The bodies ranged in size from one to fourteen microns in diameter, the majority being between three and seven microns. Those containing two or more granules were, as a rule, larger than those containing only one. In the moist spreads these bodies contained granules, dancing around in the protoplasm generally faster than the pigment of the malarial parasite. The morphology of these bodies in moist spreads and stained smears was therefore very strongly suggestive of protozoa.

The origin of these bodies, or bodies indistinguishable from them, was clearly made out. Leucocytes were very numerous in the moist spreads, particularly in those made with material from the acute exanthemata. When these spreads were watched in the warm box at 37° C., the pseudopodia of the leucocytes were seen to break off and in a short time assume a round form, each fragment containing one or more granules. When the pseudopodia which contained nuclear material had separated from the leucocytes and had assumed a regular outline, they resembled very closely individual cells. The reason the protoplasm of these bodies takes the weak basic dye instead of the acid dye is probably due to some chemical change that occurs when it separates from the cell. In some cases this protoplasm may be composed of nuclear material. In some of the stained smears leucocytes were found of which the protoplasm assumed this pale blue color, and in which the nucleus was undergoing karyorrhexis; this would indicate a degenerating cell. The nuclear fragments still gave the characteristic chromatin stain. Bodies of the same nature have been found when an emulsion of leucocytes in salt solution was left in the incubator for forty-eight hours, the salt solution having been previously diluted so as to make it hypotonic. The degenerating cells when stained gave some very beautiful pictures. (The differences in nuclear staining are shown in Plate XXVIII, Figs. 31, 32, 33, 34, and 38.)

In this connection it may be of interest to note that Gotschlich,[6] in a recent paper entitled "Ueber protozoen Befunde (Apiosoma) im Blute von Flecktyphus-kranken," describes a parasite which

he claims to be that of typhus fever. The "parasite" according to his description seems to be very similar to the bodies described by Duval in the blister fluid of scarlet-fever and by myself in the same fluid from scarlet-fever and measles. In an excellent study of the "Blood-Changes in Typhus Fever," by Love,[7] this author believes the parasite of Gotschlich to be nothing but degenerative changes in the red blood cells, and advances excellent arguments in favor of this hypothesis.

CONCLUSIONS.

I believe that the bodies found in sections of skin from cases of measles and scarlet-fever are part of the protoplasm of the epithelial cells which has been so changed in its chemical nature that its staining reaction differs from that of the surrounding protoplasm. The small round extracellular bodies found in the living patients may arise from degenerating cells, but I cannot demonstrate this origin with certainty.

In sections of control and normal skin, the nuclei of the epithelial cells were often indented by the cell protoplasm, giving them an appearance similar to those indented by Mallory's bodies.

It would seem that if these bodies of Mallory's were protozoa they would have been found in the sections from both the living and the dead skin of scarlet-fever and measles, as they were present in the blister fluid. Their absence is certainly more suggestive of a degeneration than of a protozoön. This view is also borne out by the fact that they were not found immediately after death, but were present in another specimen from the same case removed twenty-four hours later.

It would seem probable also that the bodies found in the blister fluid were the products of degeneration and cytolytic activity, because they were found in the antitoxin rashes as well as in the cases of scarlet-fever and measles.

The histological changes in the skin of these two diseases leads us to expect the presence of cytolytic products both in the blister fluid and in the sections.

It certainly cannot be stated that none of these bodies is a

protozoön, but it can be positively stated that a great majority of them arise from degenerating cells; and in many cases, I think, it is not possible to differentiate a degeneration from a protozoön by the study of its morphology and staining reactions.

The bodies present in blister fluid resemble very closely those granular bodies found in blood under certain conditions, and seen in vaccine lymph and in emulsions of tissues and in exudates. I think, therefore, that they are for the most part, if not wholly, products of degenerating tissue cells and of leucocytes, and within certain limits specific to scarlet-fever and measles.

DESCRIPTION OF PLATE.

Figs. 1, 2, 3, and 4.—Bodies in the epithelial cells from skin taken after death from scarlet-fever patients.

Figs. 5, 6, and 7.—Bodies in the epithelial cells of the skin of fatal cases of measles.

Fig. 8.—Small round bodies with the central nucleus-like point found in the skin taken from living measles patients.

A. The bodies.

B. A nucleus undergoing karyorrhexis.

C. Normal nuclei.

Fig. 9.—A lymphocyte undergoing degeneration.

Figs. 10, 11, and 12.—Polymorphonuclear leucocytes undergoing degenerative changes in blister fluid from scarlet-fever.

Figs. 13–25.—Bodies found in the blister fluid of measles.

Figs. 26–38.—Bodies found in the blister fluid of scarlet-fever.

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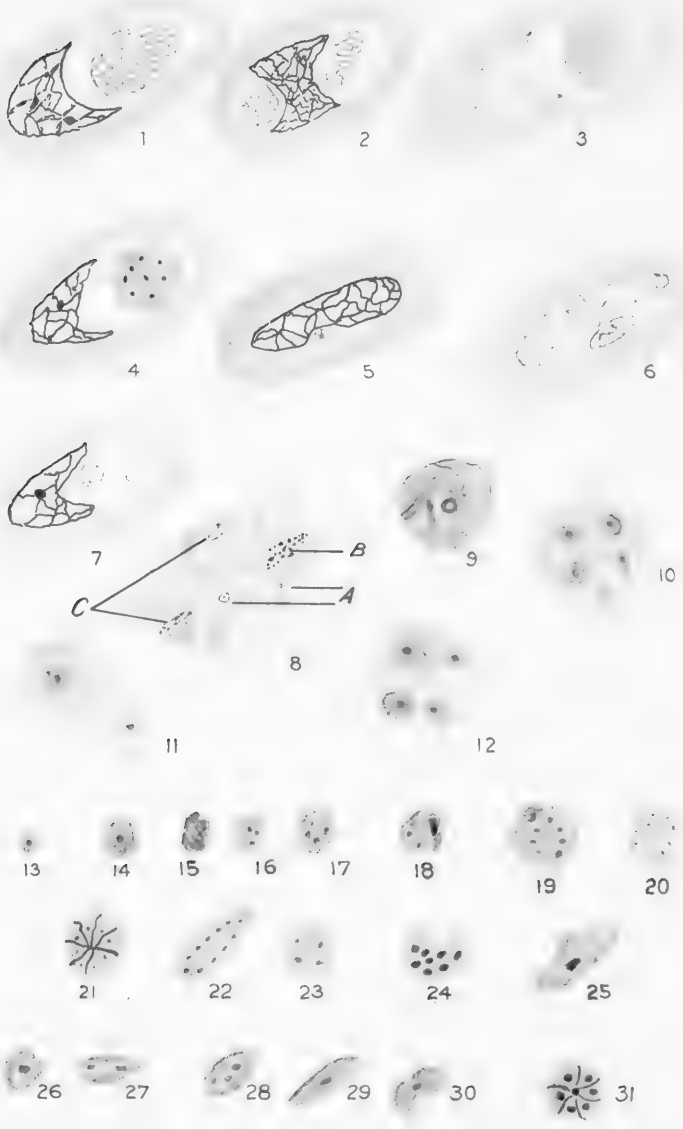
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CONCERNING THE POSSIBILITY OF AN ANTIBODY FOR THE TETANOPHILE RECEPTOR OF ERYTHROCYTES: A RECEPTOR STUDY.

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In the *Transactions of the Chicago Pathological Society*, vol. vi, p. 237, 1904, I gave a brief summary of some experiments, the object of which was to determine the ability or inability of various receptors to cause the formation of specific antibodies. We are accustomed to say that those receptors of erythrocytes with which the hemolytic amboceptors unite in the process of hemolysis or in the binding experiment, are the particular constituents of the corpuscles which cause the formation of the amboceptors when the cells are injected into the body of an animal. The amboceptors are in this sense antibodies for the corresponding receptors of the erythrocytes. Similarly, the receptors of corpuscles with which agglutinin unites, and those of bacteria with which the bactericidal amboceptors and the agglutinating bodies unite, have as their specific antibodies the hemagglutinin, the bactericidal amboceptors, and the bacterium-agglutinin respectively.

The question arises: Is this a general law? Are all bodies which function as receptors capable of giving rise to the formation of specific antibodies when they are injected into a foreign species?

The question has appeared to the writer to be one of fundamental importance, especially as it would seem to have a direct bearing on the ability of certain pathogenic bacteria to give rise to the formation of antitoxins. The body of the typhoid bacillus contains a toxic substance which we reasonably suppose to be the pathogenic constituent of the organism. Yet immunization with this toxin-containing bacterium does not

cause the formation of an indisputable typhoid antitoxin. One may assume, on the one hand, that the specific toxic substance of the typhoid bacillus is a true toxin, with a haptophorous and a toxophorous group, but that the conditions in the body are such that a specific antitoxin cannot be formed. The same argument naturally would apply to several other pathogenic organisms. Food-stuffs, and various constituents of cells are present in the body continuously, which, according to the theory of Ehrlich, may be taken up by the receptors of the cells, but for which it is thought antibodies do not develop (*horror autotoxicus*). The particular receptors with which these substances unite may not be over-produced. It is with receptors of this nature that a typhoid toxin, for example, may unite and yet not stimulate to the formation of typhoid antitoxin. On the other hand it is possible that the essential pathogenic substance of the bacillus is not a toxin in the sense of Ehrlich.

It is the purpose in this paper to present the results obtained in an attempt to demonstrate the presence or absence, in an immune hemolytic serum, of an antibody capable of uniting with those receptors of erythrocytes for which tetanolysin has an affinity. We may speak of these receptors as the tetanophile receptors of the erythrocytes; of those with which the hemolytic amboceptors unite as the serum-lysophile receptors, and of those with which serum agglutinin unites as the serum-agglutinophile receptors.

It must be frankly stated at the outset that the results obtained throw no light on the question which was proposed, yet it is thought that the conditions chosen for the experiments and the difficulties which have been encountered should be published.

If an antibody for the tetanophile receptor is formed when red blood corpuscles are injected into an animal, the process according to the theory of Ehrlich would be the following: When the erythrocytes are injected they disintegrate, are dissolved or digested, the receptors being thus liberated. The tetanophile molecules or receptors would eventually reach a

group of cells which contain molecules or receptors with which the former could unite. In accordance with the general law the latter would then be stimulated to over-production and many of them thrown into the general circulation. When the serum of the immunized animal is collected and mixed with erythrocytes of the type injected, the antibodies for the tetanophile receptors should unite with the latter as they exist in the red corpuscles. What might follow this union would depend on the character of the antibody. Inasmuch as hemolysis and agglutination are the only reactions observable when corpuscles and serums are mixed, it could not be stated definitely that an antibody for the tetanophile receptor would not be able to cause solution of the corpuscles or their agglutination. In this case the antibody would be a receptor of the second or third order, since it would be possessed of a toxophorous constituent. If, however, it were a receptor of the first order it would be passive in its nature, since such receptors possess no ferment-like or toxic action.

For the purpose of the experiments it was assumed that an antibody for the tetanophile receptor would unite with the latter and thus make impossible the anchoring of tetanolysin, just as inactive agglutinoids unite with the agglutinophile receptors of bacteria and stand in the way of subsequent union of the latter with active agglutinin. Under the assumption, the antibody in question and the tetanolysin molecule would have identical haptophore groups. It is appreciated that even if the supposed antibody is formed, difficulties might stand in the way of its demonstration by the method chosen. In the first place, the heating of the serum, which was found to be necessary in order to destroy its hemolytic power, might destroy the antibody. Again, special temperature conditions, other than those utilized, might be necessary for union. Or union having occurred, it might be an easily dissociable one; the tetanolysin might have a greater affinity for the tetanophile receptor than the antibody has, and on this account be able to displace the latter, or the union might be dissociated by subsequent washing with salt solution.

The method of experimentation consisted of exposing a definite quantity of washed erythrocytes to the action of varying quantities of the heated immune hemolytic serum for different lengths of time and at different temperatures, after which the serum was partially or totally removed by centrifugation, and the effect of tetanolysin then observed on the corpuscles. The heating of the immune serum was found to be necessary inasmuch as I did not succeed in preventing some subsequent hemolysis in the binding experiment with unheated serum at low temperatures. When such hemolysis occurred it naturally rendered an estimation of the action of the tetanolysin impossible. Normal serums used as controls were treated in the same way.

Inasmuch as the tetanophile and the serum-lysophile receptors are not identical, it was assumed that the absorption of the amboceptors did not prevent subsequent fixation of the tetanolysin by the tetanophile receptors. If the two receptors were identical, antitetanolysin should have a haptophore capable of uniting with the cytophilous haptophore of the amboceptor. Experiments showed that heated normal goat serum, rich in antitetanolysin, when added to fresh immune hemolytic serum from the goat, did not reduce the dissolving power of the latter serum. For a similar reason it was concluded that the tetanophile receptor is not identical with the agglutinophile receptor.

The first important complication which developed consisted of agglutination of the corpuscles by the serum. Although the agglutinated mass could be broken up into very fine particles by pipetting the solution back and forth, nevertheless such corpuscles often sank to the bottom readily and became reagglutinated. The mere sedimentation of the corpuscles renders them physically less accessible to the action of the tetanolysin. It is possible also that the alteration of the envelope of the corpuscle which seems to occur in agglutination renders them less susceptible to the action of a hemolytic agent. Corpuscles (guinea-pig) which have been agglutinated by ricin or abrin are to a large degree protected from the hemolytic action of saponin, although they are readily hemolyzed when distilled water is added. The smaller the amount of ricin used the greater the

hemolysis which occurs when saponin, not a true toxin, is added subsequently.

TABLE I.

THE EFFECT OF SAPONIN ON CORPUSCLES WHICH WERE AGGLUTINATED BY RICIN.

Ricin and saponin, each, 0.2 % solution; 5 % emulsion of washed guinea-pig erythrocytes in 0.85 % [1] salt solution; corpuscles and ricin at 37° for thirty minutes; ricin removed by repeated centrifugation; 0.1 c.c. saponin solution added.

Ricin.	Agglutination.	Saponin.	Hemolysis.
1.0 c.c.	Complete	0.1 c.c.	Trace.
0.5	"	"	"
0.3	"	"	Slight.
0.1	"	"	Moderate.
0.05	"	"	Marked.
0.03	Partial	"	"
0.01	"	"	Complete.
0	0	"	"

[1] Throughout the experiments 0.85 % salt solution and 1 c.c. of a 5 % emulsion of erythrocytes were used. Total volume in all tubes was 2 c.c.

Here also there may be chemical or physical changes in the envelope, or the union of hemoglobin with the stroma may be made more firm by the action of the ricin, but the physical protection of agglutination cannot be disregarded.

Guinea-pig erythrocytes which are treated with a heated immune hemolytic serum (rabbit) are also made resistant to the action of saponin, the corpuscles having been strongly agglutinated by the serum.

EXPERIMENTS WITH SAPONIN AND TETANOLYSIN.

1. Control: corpuscles were completely hemolyzed by 0.1 c.c. saponin solution in a few minutes.

2. Erythrocytes were treated with 0.3 c.c. heated immune serum for thirty minutes; washed free of serum; 0.1 c.c. saponin solution: no hemolysis. These corpuscles may subsequently be hemolyzed by distilled water.

3. Corpuscles without saponin or serum show no hemolysis.

4. Non-agglutinating normal serum gave no protection.

The following is an analogous experiment performed with the heated immune serum (rabbit), and tetanolysin:

1. Control: 0.6 c.c. of 0.2 % tetanolysin solution hemolyzed the corpuscles of guinea-pig completely in thirty minutes.
2. Corpuscles without tetanolysin or serum show no hemolysis.
3. Corpuscles treated with 0.3 c.c. serum for thirty minutes; serum removed by washing; 0.6 c.c. tetanolysin solution: no hemolysis in twenty-four hours. The corpuscles were shaken and the clot broken up repeatedly during this time. These corpuscles were subsequently hemolyzed by distilled water.
4. Non-agglutinating normal serum afforded no protection.

In view of the evident influence of agglutination as a protective condition against the action of hemolyzing substances, the protection obtained in the experiment last cited cannot logically be assigned to a preoccupation of the tetanophile receptors.

More recently experiments have been resumed using a heated hemolytic serum obtained from the goat by immunization with the erythrocytes of the sheep. A serum rich in hemolytic amboceptors, but poor in agglutinins, is obtained, as pointed out by Ehrlich and Morgenroth. It was thought that agglutination as a complicating factor might be eliminated by the use of this serum. This proved not to be the case.

Apparent protection was obtained in many experiments, even when the agglutination caused by the serum was slight, as shown in Table II. The serum (Goat 1) and blood, thoroughly mixed and diluted uniformly to 2 c.c., were placed at 37° for thirty minutes. Tubes were centrifugated, and the overlying fluid drawn off, leaving a total residue of about one twentieth of a cubic centimeter. The tetanolysin (1.0 c.c. of a 0.2% solution) was then added, each tube diluted to 2 c.c. and the corpuscles thoroughly mixed by pipetting back and forth. That the agglutinated corpuscles were largely separated in this way is shown by microscopic examination, in which no more than three to six corpuscles were grouped together. Tubes were examined after twenty-four hours.

The following experiment seems to lose significance when a duplicate, in which the serum was still further removed by an additional washing, showed that the immune serum had no, or little, more protective action than the normal serums. Yet

inasmuch as in this experiment two normal serums were used as controls, and the latter showed a lower protection than the immune serum, there must have been present some property in the immune serum, which in the normal serums was absent, or present in lower concentration.

TABLE II.

THE ACTION OF TETANOLYSIN ON SHEEP'S ERYTHROCYTES WHICH HAD BEEN TREATED WITH HEATED IMMUNE HEMOLYTIC SERUM FROM THE GOAT.

	Immune serum.	Norm. Ser. No. 1.	Norm. Ser. No. 2.	Tetanolysin.	Hemolysis.
1	0.8	...	...	1.0	No hemolysis.
2	...	0.8	...	"	Slight.
3	...	...	0.8	"	"
4	0.6	...	...	"	No hemolysis.
5	...	0.6	...	"	Slight.
6	...	...	0.6	"	"
7	0.4	...	...	"	Trace.
8	...	0.4	...	"	Slight.
9	...	...	0.4	"	"
10	0.2	...	...	"	Trace.
11	...	0.2	...	"	Moderate.
12	...	...	0.2	"	Marked.
13	0.1	...	...	"	Moderate.
14	...	0.1	...	"	Marked.
15	...	...	0.1	"	"
16	0.	0.	0.	"	Complete.
17	0.	0.	0.	0	No hemolysis.

There are two possibilities by way of explaining the loss of protection occasioned by the washing, aside from the influence of agglutination. In the first place, an antibody for the tetanolysin receptor may unite with the latter in the more concentrated

serum mixture, but the dilution involved in the washing may cause dissociation of the union, so that after the second centrifugation the tetanophile receptors may again be available for union with tetanolysin. In the second place, some other protective factor may be present in both the immune and normal serums, and in a particular experiment might be present in greater concentration in the immune serum than in the two normal serums. Antitetanolysin, or some substance of equivalent power, as cholesterin, may be present in the minute residuum of serum left in the original tubes, and it might in a given instance be present in greater quantities in the immune serum than in the normal. The corpuscles treated with the immune serum would in this instance be protected to a greater extent than those treated with the normal serum. If the serum left in the sediment after the first centrifugation is 0.008 c.c. (estimated), a subsequent washing in 2 c.c. of salt solution reduces the residual serum to a quantity which has no antitoxic effect on the amount of tetanolysin used, as shown by experiments.

Inasmuch as various quantities of serum were used in the different tubes, a higher degree of protection, which the larger amounts of serum may have afforded, might be referred to the larger amount of antitoxin present in those tubes. To eliminate this factor the amounts of residual serum in the various tubes were equalized by washing with proportionate amounts of salt solution (Table III).

Since a large proportion of the protection in A was removed by the washing, there must be present a soluble antitoxic substance in the serum.

If we assume that the inhibition of tetanolysin hemolysis in the above experiment was due to two factors, agglutination and an antitetanolytic property in the serum, and that in the washing the antitetanolysin was equalized, we have the following values for the two factors in those tubes in which 0.6 c.c. of serum was used:

Total inhibition	90 %	80 %	40 %	30 %
Antitoxin inhibition	70 %	70 %	40 %	30 %
Agglutination inhibition	20 %	10 %	0	0

These percentages are only of comparative value, inasmuch as somewhat more than the simple dissolving dose of tetanolysin was used, and at the same time the antitetanolysin in the different tubes was equalized but not entirely removed.

TABLE III.

A.—Corpuscles of the sheep exposed to action of immune serum from the goat, tubes centrifugated, overlying fluid drawn off; corpuscles not washed; tetanolysin is 1.0 c.c. of a 0.2 % solution.

		Subsequent hemolysis by tetanolysin.			
	Serum amount.	Immune Ser. No. 1.	Immune Ser. No. 2.	Nor. Ser. No. 1.	Nor. Ser. No. 2.
1	0.6 c.c.	10 %	20 %	60 %	70 %
2	0.4	10	30	80	100
3	0.2	25	60	100	100
4	0.1	75	100	100	100
5	0	Control: 100 %			

B—Duplicate of the above except that the quantities of residual serum in the different tubes were equalized by washing with proportionate amounts of salt solution.

	Serum.	Salt sol. used in washing.	Subsequent hemolysis by tetanolysin.			
			Immun. Ser. No. 1.	Immun. Ser. No. 2.	Nor. Ser. No. 1.	Nor. Ser. No. 2.
1	0.6 c.c.	2.4 c.c.	80 %	90 %	100 %	100 %
2	0.4	1.6	80	80	100	100
3	0.2	.8	100	90	100	100
4	0.1	.4	100	100	100	100
5	0	0	Control: 100 %			

The corpuscles treated with the immune serums were rather firmly agglutinated.

Nothing is deducible concerning the presence or absence of an antibody for the tetanophile receptor. Any possible protection afforded by the presence of this antibody cannot be separated from the protection due to the agglutination.

Something might be learned concerning the nature of the protection by fractional absorption of the immune bodies from the serum, and testing the action of tetanolysin on the erythrocytes which were used for the removal of the successive fractions of immune bodies. Quantities of 0.6, 0.4, 0.2, 0.1 c.c. of the different serums were diluted to 2 c.c. each, and the contents of each tube added to the sediment of 1 c.c. of the 5 % blood emulsion. After thirty minutes at room temperature the tubes were centrifugated, the overlying fluid drawn off and put into fresh tubes, while to the sediment was added the usual amount of tetanolysin in a total volume of 2 c.c. The serum dilutions were then subjected to the absorbing powers of a second mass of corpuscles of equal amount and the process repeated over four absorptions, the hemolytic power of the tetanolysin being tested on the red blood corpuscle sediments of each series. At the same time a series of tubes was made to determine the relative antitoxin content of each of the serums (Table IV, A and B).

There are three sources of error which may be responsible for some variations in the percentages of hemolysis in the different tubes and in the different series in A. First, after the corpuscles were exposed to the action of the serum and the tubes were centrifugated, the overlying serum mixtures were drawn off as completely as possible by means of a glass pipette which was drawn to a fine point; in spite of care the amounts of residual serum in the different tubes were undoubtedly subject to variations, so that Tube 1, for example, in the column headed "Immune Serum No. 1," might contain in the different series variable amounts of residual serum—that is, variable amounts of antitoxin along with other serum constituents. Second, the completeness with which the agglutinated corpuscles in the tubes treated with the immune serums were broken up, probably was not constant in spite of caution. Third, the experiment occupied a whole day and the tetanolysin lost a small percentage of its strength during that time, although it was kept on ice when not in use.

Analysis of the columns headed "Normal Serum," in which agglutination was absent, shows that although a moderate loss

TABLE IV.

A.—The effect of tetanolysin on corpuscles which have been used for the fractional absorption of antibodies from the serums.

First Series.

	Immune Serum No. 1.		Immune Serum No. 2.		Nor. Ser. No. 1.		Nor. Ser. No. 2.	
	Agglut. by serum.	Hemol. by toxin.	Agglut. by serum.	Hemol. by toxin.	Agglut. by serum.	Hemol. by toxin.	Agglut. by serum.	Hemol. by toxin.
1	Firm	10	Very firm	20	0	60	0	70
2	Less	10	Less	30	"	80	"	100
3	"	25	"	60	"	100	"	100
4	Marked	75	Firm	100	"	100	"	100
5	No serum, 100 % hemolysis: — Control.							

Second Series.

1	Gradually	15	Gradually	35	0	70	0	90
2	less from 1 to 4	20	decreased; more than	65	"	85	"	90
3		20	by Im-	100	"	100	"	100
4		75	mune Serum No. 1.	100	"	100	"	100

Third Series.

1	Gradually	10	Gradually	75	0	70	0	80
2	less from	25	less from	90	"	90	"	100
3	1 to 3	65	1 to 4	100	"	100	"	100
4	No agglut.	80		100	"	100	"	100

Fourth Series.

1	No agglut.	10	Moderate	95	0	70	0	80
2	No agglut.	15	Agglut.	95	"	..	"	100
3	No agglut.	55	Slight	100	"	100	"	100
4	No agglut.	65	"	100	"	100	"	100

B.—Antitetanolysin in the four serums.

One cubic centimeter of a 0.2 % solution of tetanolysin is mixed with the quantities of the serums stated in the table, in a total volume of 2.0 c.c. After

two hours' contact at room temperature the contents of each tube is added to the red corpuscle sediment of 1.0 c.c. of the 5 % blood suspension.

Serum	Immune Ser. No. 1.	Immune Ser. No. 2.	Nor. Ser. No. 1.	Nor. Ser. No. 2.
.005	20	50	45	60
.0045	20	60	55	60
.004	25	70	70	60
.0035	30	70	75	65
.003	35	75	75	70
.0025	45	90	90	75
.002	65	100	100	90
.0015	85	"	"	90
.001	90	"	"	95
.0005	90	"	"	100
0	Control: 100 %.			

in the protection of the corpuscles takes place as absorption continues, the degree of protection corresponds to the relative amounts of antitoxin in the two serums as shown in the antitoxin table. All, or nearly all, the protection may reasonably be assigned to the antitoxin. Inasmuch as the total volume of the sediment and residual fluid in each tube was about 0.03 c.c., the antitoxin was diluted by that much each time the serum mixture was added to a fresh lot of corpuscles. This, however, amounts to a loss of only about 0.25 % from the first to the third series and is a negligible quantity. The corpuscles also may have absorbed some of the antitoxin, a possibility which was not determined.

Analysis of the columns headed "Immune Serum," in which agglutination was present, shows a great loss of protection between the first series and the third, and the loss is more uniform and greater with the serum which had the higher agglutinating power; hand in hand with the loss of protection is the progressive decrease in the agglutinating power of the serum as absorption continues. The greater protection with Immune Serum No. 1, which was present in spite of its lower agglutinating power, is explained when we note its greater antitoxic power (Table IV, B).

This experiment, then, and duplicates which have been made, afford no clue as to the presence of an antibody for the tetanophile receptor; the protection which was encountered is explainable on the basis either of agglutination or antitoxin action.

Other experiments were performed with the view of determining more exactly the antitoxic value of the residual serum in the different tubes. The results showed that in the case of normal serums the antitoxin of the residual serum accounted for practically all of the protection, while in the case of the immune serums in which some agglutination had occurred, the calculated amount of antitoxin did not account for all the protection. The difference in protection by the two immune serums used corresponded to the differences in the agglutinating and antitoxic powers of the two serums. Here also no means developed of distinguishing an antibody for the tetanophile receptor from the agglutinating and antitoxic powers of the serums. Attempts to eliminate agglutinin by heat failed as the serums produced some agglutination even after exposure to the temperature of coagulation.

SUMMARY AND CONCLUSIONS.

The treatment of washed erythrocytes of the guinea-pig by a heated immune hemolytic serum derived from the rabbit, and of washed erythrocytes of the sheep by a similar serum derived from the goat, renders the erythrocytes more or less resistant to the subsequent action of tetanolysin.

If all the serum is removed from corpuscles treated in this manner, it can be determined that some of the protection is due to the agglutination of the cells. A certain amount of the protection afforded by agglutination is referable to the physical barrier which the agglutinated mass of cells offers to the uniform distribution and diffusion of the tetanolysin. It is probable that none of the protection obtained is due to the mere union of agglutinin or of hemolytic amboceptors with their respective cell-receptors; such union would appear to leave the tetanophile receptors still unoccupied.

If a residuum of serum is left with the corpuscles which have

been treated as indicated, the added protection which is acquired against the subsequent action of tetanolysin may reasonably be referred to antitoxin which is present in the residual serum. The possibility of the dissociation of a union between the tetanophile receptor and its antibody, occasioned by the dilution incident to washing away the serum, may not be entirely ignored, but has not been susceptible to determination. The same may be said of the possibility that tetanolysin, having a stronger affinity for the tetanophile receptor than has the antibody, is able to displace the latter from its union with the receptor.

Hence treating corpuscles with the immune serums does not allow one to determine the presence or absence of an antibody for the tetanophile receptor, the experiments being formulated on the supposition that such an antibody would unite with the tetanophile receptors and thereby prevent subsequent binding of tetanolysin.

A COMPARATIVE STUDY OF DYSENTERY AND DYSENTERY-LIKE ORGANISMS.

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Part I.—Bacilli Producing a Typical Reaction in Litmus Milk.

The general purpose of the following paper is to give an account of the many small, but perhaps important biochemical differences, which one encounters in the examination of a large number of cultures of Bacillus dysenteriæ, isolated from cases of summer diarrhœa in infants. Over one hundred cultures from twenty-two cases have been carefully studied as regards their fermentative and agglutinative properties.

During the past few years the presence of this organism has been reported in this country in several hundred of these cases, for the most part by investigators connected with the Rockefeller Institute. The aim has been to isolate this bacillus from as many cases as possible, mild as well as severe. Such success has attended these efforts that we know that bacilli resembling the dysentery organism may be recovered from even the mildest cases of summer diarrhœa in infants, if the search be sufficiently diligent and the technique good.

With a few notable exceptions, however, the organisms isolated from these cases have been subjected to tests sufficient merely to place them in the dysentery class, either within the group which does or that which does not split mannit. Bacilli of the former group have been found to occur far more frequently than those of the latter, in fact more than 90 % of the organisms that have been isolated split mannit with the production of acid. Although the mannit test is without doubt the most fundamental in the classification of dysentery bacilli, still there are a number

of minor varieties of this family which can be discovered only by employing other fermentation tests. The "Y" bacillus of Hiss and Russell and the organism splitting dextrose, mannit, and saccharose, but not dextrin and maltose, furnish examples of these variations. It seemed desirable, accordingly, to save all the cultures isolated from a series of cases for a more exhaustive examination than could be given at the time of isolation. These cultures have been brought in line as far as possible with the recent classification of Hiss ('04). He has divided the dysentery family into the following groups:

Group I.—Ferments dextrose; represented by "Shiga" culture.

Group II.—Ferments dextrose and mannit; represented by "Y" culture.

Group III.—Ferments dextrose, mannit, and saccharose; represented by "Strong" culture.

Group IV.—Ferments dextrose, mannit, maltose, dextrin, and saccharose; represented by "Flexner-Manila" culture.

Although this classification holds good in the main, a number of organisms were encountered which occupy, so far as their fermentative and agglutinative characters are concerned, intermediate positions between the last three groups; in fact one is forced to the conclusion that the mannit-fermenting forms are for the most part very unstable and capable of varying along many diverse lines.

In the recent report from the Rockefeller Institute, Duval and Shorer have noted the occurrence of mixed infection by both the mannit and non-mannit fermenting bacilli in six cases of infantile diarrhœa, and Duval and Gay found similar mixed infections in adults. Bearing this possibility in mind, careful consideration was given to the degree of diversity of all the cultures isolated from each case, which varied in number from one to sixteen.

In view of the excellent historical review which has been published by Hiss, only a few of the more recent papers which have a direct bearing on the questions in hand are referred to.

The cultures were all isolated from patients in the Sea Side Hospital of St. John's Guild on Staten Island; most of them

during the summer of 1904. I acknowledge with great pleasure my indebtedness to the members of the staff for their interest and assistance, and to Dr. P. H. Hiss for many courtesies.

GENERAL CLINICAL FEATURES.

Of thirty cases from which a dysentery-like bacillus was isolated, the dejecta in fourteen contained blood, generally in very small amount, merely a few flecks. All contained mucus in varying quantity. One stool was brown and fæcal with only the slightest trace of mucus. Nearly all the patients had been sick from one week to a month, when they were admitted to the hospital, and as a consequence in many of the cases the isolation of the bacillus was attended with considerable difficulty. Successful isolations, however, were made in about 40 % of the cases examined during the past summer. None of the patients were over two years of age and twelve were under one year. The number of stools was not large, generally from four to eight a day. The temperature in the large majority of instances was between 98° and 102° and never ran up above 104° F.

Owing to the pressure of other work it was impossible to test with any degree of completeness the agglutinating power of the patient's blood. In eight cases out of fifteen there was a good reaction at 1 : 40 with the bacillus isolated. In three instances blood, which agglutinated the dysentery bacillus, was tested on several motile gas-producing organisms and on a slowly motile organism not producing gas, isolated from the same stool, but positive results were not obtained in any case. A number of dried specimens of blood, which were reserved for future work, were found after about two months to have lost entirely all clumping power. One specimen which agglutinated the dysentery bacillus very rapidly at 1 : 50, when first obtained, was included among those tested at a later date. The agglutinins for the dysentery bacillus thus appear to be less stable in the patient's blood than in the case of typhoid fever.

By far the most interesting case encountered was one of an infant twenty months old from which the true "Shiga-Kruse" organism was obtained in large numbers; death occurred nine days after the beginning of the attack. Fifty-eight isolations were tested and all were found to be the non-mannit fermenting ("Shiga-Kruse") variety. On one plate of about 200 colonies, 55 % were found to be Bacillus dysenteriæ (Shiga). In view of the fact that from only 10 % of all the cases reported by the Rockefeller Institute the "Shiga-Kruse" type was recovered, its presence in such numbers in this case is worthy of note. Nearly pure plates of the "Flexner-Manila" type have been obtained by Duval, Wollstein, and others, but in those cases in which the "Shiga-Kruse" type has been found to be the probable etiological cause, the colonies have been comparatively few in number.

Among the organisms isolated from this case was a slowly motile one, clouding slightly the semi-solid medium. This culture had all the characteristics of the dysentery bacillus, reacting typically in litmus milk and agglutinating in high dilu-

tions with the blood of a rabbit immunized to Group I. Motile dysentery bacilli have been noted by Duval as occurring among his cultures isolated from infants and, in fact, motility was one of the characteristics originally given by Shiga and Flexner to the dysentery bacillus. Kruse described his original culture as non-motile and it has been determined within recent years that the vast majority of cultures lack motility, although Duval and Vedder demonstrated flagella by a modified staining technique, and Duval and Bassett succeeded in developing motility by special treatment. Dunn has recently described a motile and probably mannit-splitting culture.

METHODS.

The technique described in the recent Report of the Rockefeller Institute was followed in its main essentials. In some instances plates were made with Hiss medium alone or in addition to agar. Much greater success was obtained by the use of ordinary slightly acid nutrient agar than with Hiss plate medium. The number of plates averaged about ten to a case. Suspicious colonies were transplanted in Hiss semi-solid medium and all motile and gas-producing organisms were discarded. In a number of instances the production of gas could alone be detected by stirring the medium, especially in the case of organisms, which were not able to spread through the jelly. The non-motile, non-gas-producing cultures were then planted in litmus milk and in Hiss litmus-serum water, to which had been added one per cent. of mannit. Finally, twenty-four-hour broth cultures were tested with an anti-dysenteric serum by the hanging-drop method, and where possible with the blood of the patient.

BIOCHEMICAL DIFFERENCES IN THE DYSENTERY ORGANISMS ISOLATED.

Fermentation Tests.—As indicated above, 100 cultures, which did not split lactose, were selected for thorough tests of their fermentative activity. Five sugars were employed as follows: the monosaccharid, dextrose; the disaccharids, maltose, saccharose, and lactose; and the polysaccharid, dextrin. These were added in the proportion of one per cent. to the litmus-serum water of Hiss. Lactose was not used in those cases where extended tests had already been made with litmus milk. It is very important that the serum medium be clear

and sterilized not longer than ten minutes at a time. The different sugars were repeatedly tested with stock cultures of the dysentery bacillus whose fermentative reactions are well known. Merck's highly pure maltose was used. The criterion for all the sugars, however, was their reaction with well-known stock cultures and not the name of the manufacturer. The cultures were incubated at 37° and were observed at intervals of two or three days for two weeks. Where it was deemed advisable they were kept under observation for a month. Before being tested each culture was subjected to a preliminary cultivation in broth or on agar for a few days. All of the principal types and varieties disclosed by the first test were retested after being "standardized," according to the treatment suggested by Fuller and Johnson, i. e., three days' growth in broth, three days' on gelatine plates, and three days' on agar. In order to avoid making further artificial groups, all cultures which split four sugars were classified under Group IV, although the distinguishing characteristic of this group, as given by Hiss, is the splitting of five sugars. This plan of putting the more active fermenters together in one group has been followed in a number of recent articles. The classification in the following table is based on a four- or five-day test, since, as has been pointed out by Hiss ('04), the fermentative differences between the several groups are best observed at this time.

TABLE I.

Fermentation Tests.

Case Number.	Number of Colonies tested from each case.	Dextrose.	Mannit.	Maltose	Saccharose.	Lactose.	Dextrin.	Group according to Hiss.
1	9	R & C	—	—	—	—	—	Group I
2	10 { 9	R & C	R & C	—	R	—	—	Group III
	10 { 1	R & C	R & C	R	R	—	—	Group IV
3	1	R & C	R & C	—	—	—	—	Group II
4	13 { 9	R & C	R & C	—	R	—	—	Group III
	13 { 4	R & C	R & C	R&C	P	—	R	Group IV
5	16	R & C	R & C	—	R	—	—	Group III
6	3	R & C	R & C	—	R	—	—	Group III
7	3	R & C	R & C	—	—	—	—	Group II
8	11	R & C	R & C	—	—	—	—	Group II
9	4	R & C	—	—	—	—	—	Group I
10	1	R & C	—	—	—	—	—	Group I
11	1	R & C	R & C	R	R	—	—	Group IV
12	3	R & C	R & C	—	—	—	—	Group II
13	10 { 4	R & C	R & C	—	R	—	—	Group III
	10 { 2	R & C	R & C	R & C	R	—	P	Group IV
	10 { 2	R & C	R & C	—	R	—	P	Group IV
	10 { 1	R & C	R & C	R & C	R	—	—	Group IV
	10 { 1	R & C	R & C	—	—	—	—	Group II
14	2	R & C	R & C	—	R	—	—	Group III
15	0	R & C	R & C	—	—	—	—	Group II
16	3	R & C	R & C	—	—	—	—	Group II
17	4	R & C	R & C	P	R	—	—	Group IV
18	2	R & C	R & C	—	—	—	—	Group II
19	1	R & C	R & C	—	—	—	—	Group II
20	2	R & C	R & C	—	—	—	—	Group II
21	1	R & C	R & C	—	..	—	—	Group II
22	1	R & C	R & C	—	..	—	—	Group II

R & C stands for reddening and coagulation of the medium.
R " " reddening of the medium, no coagulation.
P " " slight evidence of acidity.

The foregoing table shows that after four days' growth in the various sugars at 37° C., the cases may be divided as follows:

Group I	3 cases
Group II	11 cases
Group III	7 cases
Group IV	4 cases

Especially noteworthy is the fact that in only two cases were cultures obtained which acted on dextrin within four days, being thus in accordance with the Group IV of Hiss. The dextrin, and in fact all the sugars, as has already been stated, were repeatedly tested with the well-known cultures, "Baltimore," "Y," and "Kruse," the results corresponding entirely with those obtained by other investigators. Organisms belonging to Group III always produce acid early and quite strongly in saccharose and attack maltose, if at all, only after several weeks' cultivation. Cultures from two of the cases acted very strongly on saccharose, in a few instances reddening and coagulating the medium in four days.

Only two other attempts have been made to classify in three or more groups the cultures obtained from a series of cases. The recently published results of Weaver and Tunnicliff correspond quite closely with my own, although they encountered no cases belonging in Group I. Eleven of their cases yielded bacilli of the "Flexner-Harris" type and seventeen of the "Y" type. They used the classification of Park into three groups, organisms fermenting three sugars being put in Group II. From a study of their table it seems probable that, according to Hiss' classification, their cases might be arranged as follows: ten in Group II, seven in Group III, and eleven in Group IV. Of the Group IV cultures, only two react on the sugars exactly like the "Flexner-Harris" bacillus. Some do not split maltose, others have no effect on dextrin, and still others none on saccharose. They obtained entirely typical results in testing their media with the stock cultures "Y" and "Flexner-Harris." According to Duval and Schorer, the "Flexner-Harris" type far outnumbers the "Y." Using dextrin alone as a means of differen-

tiation, they found that fifty-five cases of summer diarrhœa were due to infection with the "Flexner-Harris" type (in eleven instances associated with "Y"), one to "Y" alone, and eleven to "Shiga-Kruse" type alone. It may be said, however, that dextrins of various makes differ very much in quality, sometimes being little better than dextrose.

The nearest approach, in my series, to a mixed infection is found in Cases 2, 4, and 13. In Case 2, nine cultures produced the typical reaction of Group III and one the reaction of Group IV. Again, in Case 4 we find the same combination, nine belonging in Group III and four in Group IV. Apparently in Case 13 we have representatives of Groups II, III, and IV. The culture, however, which after a four-day test belonged in Group II, had after seven days reddened saccharose, and thus, if the time limit had been placed a few days later, it must have been placed in Group IV. Weaver and Tunnicliff similarly found only two cases in which organisms from two or more groups were associated; in one there were bacilli of Groups II and III and in the other of Groups III and IV. Furthermore, in making test with immune sera for agglutinins, I found that dysentery organisms from the same case have similar receptors, and in spite of slight differences in the power of splitting sugars are fundamentally identical organisms. That we may find mixed infections with the two main divisions—the "Shiga-Kruse" and the "Flexner-Manila"—has been proven, as has already been remarked, by Duval and Gay in adult cases and by Duval and Schorer in infantile cases. The fact that they have been reported by no other investigators indicates that either they must be very rare or that too few organisms have as a rule been isolated and tested from the cases.

Case 17 is of peculiar interest in this connection. When first tested in the sugars about two months after isolation, one of the cultures apparently belonged to Group I as it acted only on dextrose, while the other three gave the reactions of Group III. This was still the case after thirty days of growth. The first culture was transplanted a number of times on agar and retested in mannit, but with the same result. When, however, a culture

of this organism was taken on agar from the mannit tube and then replanted in mannit the medium was reddened and coagulated. Although there is no doubt about the correctness of the observations of Duval and Gay, since their results were controlled by agglutination experiments, still the result given above is certainly suggestive of a possibility of error of interpretation in the determination of mixed infections.

Reactions in Litmus Milk.—The reaction of the dysentery bacillus in litmus milk is of great importance in its identification and has been repeatedly described. When first isolated, the different groups act on the medium in an almost identical manner. After the initial acidity there is in a few days a return to the amphoteric and finally to a decided alkaline reaction. Under cultivation, however, considerable differences in the reaction arise, which is merely another instance of the variable and unstable nature of the group of mannit fermenters. Group II turned to alkalinity in three or four days, whereas Group IV ("Baltimore") did not reach the same stage until seven to eleven days. Both produced a marked alkalinity in eleven to eighteen days. A number of organisms, however, which in their fermentative reactions belong to Group II or III, to the latter especially, do not change the reaction of the medium at all even during thirty days' growth. Several cultures belonging in Group I, which when first isolated were entirely typical in their effect on litmus milk, did not produce, about six months later, a marked alkaline reaction. After returning to amphoteric there was no change to alkalinity, but if anything the medium appeared slightly more acid than the control after three weeks' growth.

Reaction of Indol.—In the production of indol we find great variation. Group IV produces it most strongly, Group III may or may not produce it, and the same may be said of Group II. Group I never produces it. As a more vigorous growth resulted in peptone-serum water than in Dunham's peptone solution, this medium was largely used. The cultures which split maltose vigorously generally produce indol, as has been noted by Hiss and Park. It was found that by transplanting in the medium at intervals of three days, an organism might be induced to produce indol quite strongly which at the first test either did not produce it at all or very weakly. This was especially true of "Y." The strongest production of indol was always at the third transplantation. At the fourth there was considerable weakening, and by the fifth or sixth transplantation most of the organisms had died out. Peckham has also found that any typhoid culture may be induced to give rise to indol, the greatest production taking place at the seventh day of the second planting. At the best the production of indol is a variable characteristic; one of two cultures, exactly alike in their fermentative activities, produces it, while the other does not. Cultures, too, from the same case may produce indol in a different degree regardless of the presence or not of slight fermentative differences, although they all agglutinate alike. Other instances showing the unstable and variable tendencies of the mannit-fermenting bacilli might be given.

Artificial Varieties.—A number of attempts were made to change the fermentative character of the various groups with indifferent success. It was found that the power to split maltose could be greatly increased by cultivating the organism alternately anaërobically and aërobically. This was especially the case with "Kruse." A culture was finally obtained which reddened and coagulated maltose-litmus-serum water in two or three days. This "acquired" character persisted with each transplantation. The action of "Baltimore" on lactose and "Y" on maltose was also increased by this treatment. In spite of many attempts, however, the culture "Kruse" could not be induced to act on mannit, although, as we have seen, a member of Group III may lose its power to split mannit. Weaver and Tunnicliff have noted a variability of the same organism in the fermentation of sugars—some gained and a few lost under cultivation. They think that the reaction on saccharose, maltose, and dextrin is not constant, but may vary according to the conditions under which the organisms have been growing. By plating out a culture and then comparing the fermentative power of the colonies with that of the original culture, Hiss found often a marked increase in the cultures obtained from the plates. The writer also observed that after subjecting several colonies to a three days' growth on gelatine plates, then in broth, and finally on agar, there might be change in the fermentative power. Out of fifteen cultures so treated there was an increase in five, a decrease in six, and no change in four. The only variations were in regard to maltose, dextrin, and saccharose. The most plausible explanation seemed to be that, especially among cultures splitting three or four sugars, there are variants either in the direction of Group II or Group IV, and the result obtained depended on the colony that was selected for the test. This conclusion was further strengthened by the fact that in transplanting from one culture into several tubes of the same lot of medium there might be slight differences in the degree of fermentation. The increased action of "Kruse" on maltose, and especially the permanence of this increase, may be explained by this hypothesis, for under anaërobic conditions those individuals

which could obtain their oxygen by the splitting of maltose would soon crowd out their less fortunate competitors, and the culture would tend to become purely maltose fermenters.

AGGLUTINATIONS.

Normal Rabbit Serum.—It has been shown by Park, Bergey, and others that the normal serum of the horse and even that of smaller animals possess a considerable amount of normal agglutinin for dysentery bacilli. In testing the blood of seven normal rabbits, I found that the strain "Baltimore" clumped with all in dilutions of from 1:40 to 1:200. This indicates, of course, a possibility of error in results that have been based on dilutions of immune serum lower than 1:200. It will be seen in the following table that "Y" reacted with none of the normal sera—that is, in dilutions as low as 1:10 or 1:20. Another organism (Case 7), whose fermentation reactions place it in the "Y" group (II), reacted in about the same dilutions as "Baltimore" (Group IV); but it will be shown in a subsequent table that this culture agglutinates with immune sera almost exactly like "Baltimore" and is nearly related to that organism. Cultures belonging in Groups I and III, that were tested, did not react with any of the normal rabbit sera.

TABLE II.

Normal Rabbit Sera.

CULTURES:	Rabbit I.	Rabbit II.	Rabbit III.	Rabbit IV.	Rabbit V.	Rabbit VI.	Rabbit VII.
Group I "Kruse".....	0	0	0	0	0	..	..
Group II "Y".....	0	0	0	0	0	0	0
Group II, Case 7.....	..	20	..	40	0	..	0
Group III, Case 2.....	0	0	..	0	0	0	0
Group IV, Case 4.....	40	..	..	40	40	..	0
Group IV "Baltimore"....	40	40	100	40	40	200	100

Immune Rabbit Serum.—Rabbits were immunized to representatives of each of the four groups. For these experiments eight animals were used. The inoculations were given first subcutaneously and then intraperitoneally. Twenty-four-hour agar cultures suspended in normal salt solution were always employed. To some of the rabbits cultures heated to 62° C. for a half hour were given at first, and after they had received four whole cultures extending over a period of about four weeks the blood was drawn and tested. Four living cultures were then given and the blood was tested again. As controls, living cultures were given from the start to three rabbits. A comparison

shows that the chief, specific or major agglutinins run higher with inoculations of living cultures than with heated ones, but that the common or minor agglutinins are present in practically the same degree in both. There is, accordingly, a greater difference between the major and minor agglutinins with the living than with the heated culture inoculations. The serum of a rabbit to which heated and then living cultures were given clumped the various groups in almost an identical manner with that of a rabbit which had received living cultures from the first. None of the rabbits died and only the one immunized to Group I showed any signs of sickness. This rabbit after the first subcutaneous injection of one-fiftieth of a heated agar culture became very sick, lost weight rapidly, and suffered a partial paralysis of the posterior extremities. After complete recovery had taken place, however, the second and larger dose produced no sickness, and soon whole living cultures could be given intraperitoneally without untoward results. The care, the feeding, and the time of the year have a great deal to do with the resistance of rabbits to inoculations with dysentery bacilli, fall and winter being far more favorable than spring and summer.

In testing the sera, both formalized and fresh emulsions in normal salt solution of twenty-four-hour agar cultures were used. It was found that there was no appreciable difference in the reactions of the two, as far as the relation of the major to the minor agglutinins was concerned. As formalized emulsions can be kept in considerable quantity and one can be sure that the dilutions are always to the same degree, they are more satisfactory and safer than living emulsions. Accordingly, they were largely employed in the following experiments. On account of the normal agglutinins present in rabbits' blood the dilutions were always above 1 : 100. In the tests, 1 c.c. of culture emulsion was added to 1 c.c. of the diluted serum in a small test-tube. These tubes were placed in the incubator for three hours and then left at room temperature until the following day. Readings were made with the aid of a hand lens at the end of one, two, three, and twenty-four hours. When, at the end of twenty-four hours there was a decided deposit in the tube and the clumps in suspension were large enough to be seen with the unaided eye, the reaction was called positive.

In a recent paper by Park and Collins, the relation of specific to common agglutinins has been discussed. They find that during the first two weeks the specific agglutinins are practically the only ones present, but after three or four weeks the common agglutinins begin to increase in quantity and may almost equal the specific in amount. Obviously it is important to bear this variation in mind in making a comparative study of agglutination, and the serum tested should be drawn from the several rabbits after the same length of time has elapsed and the same amount of culture has been given. The figures in the foregoing table are compounded from a large number of tests and, I think,

TABLE III.

Immune Sera.

Cultures.	Rabbit immunized to Group I, Case 1.	Rabbit immunized to Group II "Y."	Rabbit immunized to Group III, Case 2.	Rabbit immunized to Group IV "Baltimore."	Rabbit immunized to Group IV, Case 4.	Rabbit immunized to B. Typh.	Rabbit immunized to B. Paratyph.	Rabbit immunized to B. Coli.
Group I "Kruse."	10,000	0	0	0	■	0	0	0
Group I, Case 1	Homologous 10,000	■	0	200	0	0	0	0
Group I, Case 1–17, motile	2,000	0	0	0	0	0	...	...
Group I, Case 9	10,000	0	0	200	0	0	...	...
Group I, Case 10	10,000	0	0	200	0	0	...	0
Group II "Y"	■	Homologous 6,000	0	1,000	0	200	0	0
Group II, Case 16	0	12,000	0	1,000	0	200	...	...
Group II, Case 7	500	2,000	10,000	15,000	500	1,000	0	0
Group II, Case 3	100	200	400	1,000	3,000	200	..	..
Group II, Case 12	500	200	500	2,000	1,000	0	..	0
Group III, Case 2	200	0	Homologous 8,000	1,000	500	0	0	0
Group III, Case 14	0	12,000	500	1,000	200	...	..	..
Group III, Case 5	100	200	500	2,000	4,000	100	...	0
Group IV, Case 17	100	1,000	2,000	15,000	400	500	0	0
Group IV "Baltimore"	500	2,000	1,000	Homologous 15,000	1,000	1,000	100	100
Group IV, Case 4	100	100	500	1,000	Homologous 4,000	200	200	100
Group IV, Case 13	200	200	10,000	2,000	1,000	0	...	■
Group IV, Case 11	0	■	10,000	1,000	200	0	...	0
Typhosus B	0	200	200	200	100	Homologous 40,000	1,000	0
Paratyph. B	100	200	0	100	100	100	Homologous 40,000	■
Coli B	100	100	100	200	100	200	0	Homologous 100,000

represent the true relative agglutinative power in each case. With the exception of the Group I rabbit, the relation of the specific or major to the common or minor agglutinins remained practically the same from six to twelve weeks, or until nine cultures had been given. The rabbit immunized to Group I, however, lost nearly all of the minor agglutinins during that period.

A glance at Table III shows clearly that the "mannit-fermenters" differ markedly in the way in which they agglutinate with the various sera. This may be the case even with cultures which act on the various sugars in an identical manner. Taking as an example Case 7, Group II, we see that it agglutinates in a manner very similar to that of "Baltimore" Group IV. The same may be said of Case 17, Group IV, which does not split dextrin. Again, Case 3, Group II; Case 5, Group III; and Case 4, Group IV, react with the various sera in practically an identical manner. This indicates that many intermediate forms exist between the various groups, and also that from the fermentative activities one cannot infer the agglutinative activities of a "mannit-fermenter." Case 2, Group III, and Case 4, Group IV, although their sugar-splitting powers are quite similar, raised agglutinins which were entirely different from one another, and also different from any that have been described heretofore. In Case 14 we evidently have an erratic organism identical with the "Ferra" culture described by Hiss. It belongs in Group III, if the splitting of sugar be taken as a criterion of species, but it agglutinates in practically an identical manner with "Y." Case 1–17, Group I, was motile when first isolated. It will be seen by the table that it is a poor agglutinator, and clumps only with Group I serum. Another poor agglutinator is Case 12, Group II; the limit of reaction is so low with all the sera that only absorption experiments can indicate which holds its specific agglutinins. This organism was subcultured a number of times without increasing its agglutinability. In Case 11, Group IV, we have a culture which agglutinates very well with its specific serum (Group III), but very poorly or not at all with the other sera. Several cultures from Case 2, although differing

slightly in their fermentative powers, agglutinated alike, and the same was true of cultures from Case 4 and Case 13. Typhoid serum of a high titer agglutinated cultures similar to "Baltimore" as high as 1:2000 and "Y" up to 1:500, as has been shown by Hiss. Groups I and III did not react with typhoid serum above 1:100. Paratyphoid and colon bacilli evidently raise fewer common agglutinins for dysentery bacilli than does the typhoid bacillus. Various dysenteric sera contained few or no agglutinins for typhoid, paratyphoid, and colon bacilli. Whatever ones were present may probably be considered normal to rabbits' blood.

Absorptions.—Agglutination of bacteria in high dilutions of a serum does not necessarily indicate specific relationship, but the ability to absorb all or a large part of the specific or major agglutinins from such a serum is regarded as the true test of relationship. The following absorptions are selected from a considerable number, which were undertaken with the idea of finding the relationship of some of the "erratic" organisms to the various groups of dysentery bacilli. The technique described by Hiss was followed in its main essentials. No tests were made below 1:200 for reasons which have already been stated.

Absorptions of all the sera with the culture Case 7, Group II, gave some interesting results.

GROUP I SERUM ABSORBED WITH CULTURE GROUP II, CASE 7.

Cultures.	After absorption.	Before absorption.
Group I, Case 1	10,000	10,000
" II, " 7	0	200
" III, " 2	0	200
" IV "Baltimore"	200	500

None of the major agglutinins were taken out, but part of the minor agglutinins for "Baltimore" and all for Group III were removed. This absorption indicates that there is no specific relationship between this culture and Group I, but that the minor agglutinins which cause its agglutination in Group I serum also are concerned with that of Group III cultures, and in part with that of Group IV cultures.

GROUP II "Y" SERUM ABSORBED WITH CULTURE GROUP II, CASE 7.

Cultures.	After absorption.	Before absorption.
Group I, Case 1	0	0
" II, " 7	0	2,000
" II "Y"	6,000	6,000
" II, Case 3	0	200
" III, " 17	0	1,000
" IV, " 13	0	200
" IV "Baltimore"	200	2,000

None of the major agglutinins were removed, but nearly all the minor, all for Cases 3, 17, and 13, and a large part of those for "Baltimore."

GROUP III, CASE 2, SERUM ABSORBED WITH CULTURE GROUP II, CASE 7.

Cultures.	After absorption.	Before absorption.
Group II, Case 7	0	2,000
" III, " 2	2,000	8,000
" II "Y"	0	0
" IV "Baltimore"	0	1,000
" IV, Case 17	5,000	10,000

The major agglutinins are considerably reduced in amount and the minor are entirely removed.

GROUP IV "BALTIMORE" SERUM ABSORBED WITH CULTURE GROUP II, CASE 7.

Cultures.	After absorption.	Before absorption.
Group II, Case 7	0	15,000
" IV "Baltimore"	500	15,000
" IV, Case 17	0	15,000
" II "Y"	0	1,000
" III, Case 2	0	1,000
" IV, " 4	0	1,000

The major agglutinins were very much reduced in quantity and all the minor were removed. It took no less than twenty

agar cultures to remove the agglutinins from ½ c.c. of serum used in the last experiment.

From the above absorption experiments it is clear that the culture Case 7, Group II, bears no specific relationship as far as agglutination is concerned to "Y" Group II, as it absorbs none of the major agglutinins of "Y" serum. It reduces very largely the major agglutinins of Group IV "Baltimore" serum, and also to a considerable extent those of Group III, Case 2, serum. From every serum it takes out all the minor agglutinins. This is, then, a very generalized organism. It possesses some of the characteristics of all the "mannit-fermenters," but is most nearly related to Group IV.

In showing that organisms, acting in a different manner on the various sugars, may have an agglutinable substance which is identical, the following experiments are instructive:

GROUP IV, CASE 4, SERUM ABSORBED WITH CULTURE GROUP III, CASE 5.

Cultures.	After absorption.	Before absorption.
Group III, Case 5	0	4,000
" IV, " 4	0	4,000
" IV "Baltimore"	0	1,000
" II, Case 3	0	3,000
" II, " 7	0	500

SAME SERUM AS ABOVE ABSORBED WITH CULTURE GROUP II, CASE 3.

Cultures.	After absorption.	Before absorption.
Group II, Case 3	0	3,000
" IV, " 4	0	4,000
" III, " 5	0	500
" IV "Baltimore"	0	1,000

All the major and minor agglutinins are removed, accordingly organisms belonging to the three groups of mannit-fermenters may have identical agglutinable substances.

The following absorption shows that Case 4, Group IV, and

"Baltimore" Group IV are entirely distinct agglutinating species.

GROUP IV, CASE 4, SERUM ABSORBED WITH CULTURE GROUP IV "BALTIMORE."

Cultures.	After absorption.	Before absorption.
Group IV "Baltimore"	0	1,000
" IV, Case 4	4,000	4,000
" II, " 3	3,000	3,000
" III, " 5	4,000	4,000

The culture "Baltimore" affects neither the major nor minor agglutinins of Group IV, Case 4. Active fermenters of the dysentery group may, accordingly, bear no relationship to one another as far as agglutination is concerned.

That there is sometimes a partial relationship between members of Group IV is shown by the following absorption:

GROUP IV "BALTIMORE" SERUM ABSORBED WITH CULTURE GROUP IV, CASE 13.

Cultures.	After absorption.	Before absorption.
Group IV, Case 13	0	2,000
" IV "Baltimore"	5,000	15,000
" III, Case 17	2,000	15,000
" IV, " 4	200	1,000
" II, " 7	5,000	15,000
" II "Y"	200	1,000

In all instances where the fermentation and agglutinative reactions did not correspond, the cultures were retested in the sugars after the preliminary cultivation that has been described hitherto. In the instance of Case 7, Group II, which simulates so closely the agglutinations of "Baltimore" Group IV, this was done twice, but the bacillus never split any sugars other than dextrose and the alcohol mannit. It seems evident, then, that organisms which manifest identical physiological activities may have a quite different receptor apparatus, and, on the other hand, that organisms with identical receptors may act differently on the various sugars. There is no necessary

connection between the two. Durham has found the same to be true in the B. coli group. Exactly similar cultures failed to interagglutinate, whereas one culture which failed to ferment saccharose interagglutinated in very high dilutions with one which fermented that sugar. We know now, however, that absorption is the only reliable criterion of specificity. Weaver, Tunnicliff, and Heineman have recently published some peculiar results as far as the agglutination of a series of dysentery cultures is concerned. Their results are, I think, to be ascribed largely to the fact that the titer of their various serums was very low, only 1:200, and that they used broth cultures for inoculations. Park and Collins have recently shown that broth alone may give rise to agglutinins for maltose-fermenting organisms of the dysentery group. That fact taken in connection with the normal agglutinins present in rabbits accounts, I think, for the lack of harmony between their results and those recently published by Hiss and Park, and also my own. Heineman and his associates found that several cultures agglutinated with their Group I serum alone. It is well known, however, that Group I serum agglutinates the mannit fermenters, if at all, in low dilutions, and that the major agglutinins take no part in this reaction. The agglutination of "Y" itself in the Flexner serum is entirely different from what has been described by Hiss and Park. Although "erratic" organisms do occur, they are not so numerous as we would be led to conclude from their tables. I have not found the statement of Park and Carey to hold, that all dysentery organisms producing indol in large amount and fermenting mannit agglutinate alike, for many representatives of Groups III and IV, which produce indol strongly, may have little in common in their agglutination.

GENERAL CONCLUSIONS.

The group of dysentery organisms is a large and varied one. It may be divided, as is well known, into two main sub-groups, the "Shiga-Kruse" and the "mannit-fermenters," which are of equal importance and entirely distinct. If we take decided differences in agglutination, as determined by absorptions, as a

criterion of specificity, the latter group includes at least four distinct species with a number of sub-varieties and transition forms. Two of the above species are found among organisms which split either dextrose, mannit, and saccharose or in addition maltose. The other two species are represented by the bacillus "Y" of Hiss and Russell and the "Flexner-Manila" bacillus. If we adopt Ford's proposal, that a new species should be made for each culture showing a constant cultural or agglutinative difference, the number would increase considerably. At the best, however, classification of bacteria is at present very artificial. Although so many types appear among the "mannit-fermenters," there is no reason why they should not all be included in the dysentery group of bacilli, provided the reaction in litmus milk be typical. This cultural test, as Duval has emphasized, is the most constant one which we have at present, but it should extend over several weeks in order to exclude lactose fermenters.

In contradistinction to the heterogeneity of the "mannit-fermenters," the homogeneity of the "Shiga-Kruse" type stands in marked contrast. Every culture of the latter which was tested (about twenty in all) reacted in all media and agglutinated with all the various sera exactly alike. One is tempted to explain this diversity of the "mannit-fermenters" by accepting the suggestion of Flexner, that they may be occasional, if not constant, inhabitants of the normal intestine. Certainly Duval has isolated the "mannit-fermenters" from the mildest cases of diarrhœa, to say nothing of the two apparently normal infants from which he also obtained them. Furthermore, the agglutinins for the mannit group in normal blood might be accounted for on this hypothesis, as well as the many differences in fermentation and agglutination. For, as has been well said by Smith and Reagh, one "should keep in mind the various adaptations in the intestine—it may be to the food remains in the large intestine, to the mucus on the surface of the epithelium, to the contents of the tubules and the larger flask-shaped glands," and further, "with this progressive adaptation are associated modifications of biological characters which most likely involve

agglutinative capacities as well." The fact that dysentery bacilli have not been isolated frequently from the normal intestine might be explained by their habitat being possibly within the mucus tubules and their number few under normal condition, for it is well known that they are most frequently found in association with mucus. When abnormal conditions, however, arise and the production of mucus increases, these organisms would possibly multiply in number *pari passu* and find themselves in a favorable position to attack any slight injury which might occur in the wall of the intestine.

The "Shiga-Kruse," or Group I, type, on the other hand, seems to present the characters of a true parasite. Only an insignificant amount of agglutinin is present for it in normal human blood and that of laboratory animals, according to most authorities; it agglutinates alike in various immune sera; it has a very marked toxicity for laboratory animals; it has never been isolated from the normal human intestine, although an organism in some respects like it has been described by Ford as being an inhabitant of the normal intestine; it produces no indol; it splits only the monosaccharids, through which it falls in line with the conclusion of Smith and Reagh, that the less a bacillus acts on sugars the more pronounced is its parasitic character. Whether or not it ever invades the blood in numbers is a question yet to be decided.

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A COMPARATIVE STUDY OF DYSENTERY AND DYSENTERY-LIKE ORGANISMS.

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Part II.—Dysentery-like Organisms which Produce an Atypical Reaction in Litmus Milk and which should probably be Included within the Group of Pseudo-Dysentery Bacilli.

From time to time, within recent years, several varieties of Bacillus dysenteriæ have been described, which differ from the original culture of Shiga in their action on various sugars and in agglutination. In every other respect, however, and especially in the changes produced in litmus milk, they are practically identical with Shiga's bacillus. All of these, according to the evidence available at present, have an equal claim to be included within the group of dysentery bacilli. On the other hand, there are other dysentery-like organisms whose status has not been established. They differ from the dysentery bacillus most noticeably in their action on litmus milk and in their failure to agglutinate in high dilutions of any antidysenteric serum. On the grounds which will be indicated in this section, it seems best to the writer to include all of these divergent forms within the group of "pseudo-dysentery" bacilli. Their very marked resemblance to the bacillus of dysentery, however, renders desirable a detailed description and an attempt to determine the degree of their relationship to the dysentery bacilli group on the one hand and to the typhoid and colon bacilli groups on the other. Accordingly, a considerable number of cultures obtained during the past summer were reserved for study.

A few of these isolations so closely resembled at first the type

of "mannit-fermenters" of the dysentery group that it was only after a cultivation in litmus milk for two or three weeks that their true character was revealed. One large group, which splits lactose after a longer or shorter period of growth in litmus milk or Hiss' lactose-litmus-serum water, do not produce gas, are not motile, do not liquefy any medium, and produce no change in neutral red-glucose agar. In morphology they cannot be distinguished from the dysentery bacillus. In Hiss' semi-solid medium the growth is also very similar to that of B. dysenteriæ, with the exception of a slightly greater spreading over the surface. There is not, on the other hand, the slightest spreading through the medium. This tendency to spread is also seen on agar slants and on agar and gelatine plates. In gelatine they form the familiar "grape-leaf" colony. Indol was not produced by any of these cultures, but all reduced nitrates to nitrites.

The occurrence of these organisms in cases of summer diarrhœa in infants was first noticed by Duval and Schorer, and a short description is given in their article in the studies from the Rockefeller Institute.

The stools from which the writer's cultures were obtained were generally yellow-green muco-fæcal in character and seldom contained even a trace of blood. Although several of these infants from whom the stools were obtained died, there is no proof that the bacilli in question were an etiological factor in the cause of their disease, especially as in the few instances in which tests could be made the patient's blood either failed to agglutinate the bacilli or did so only in low dilutions. In no case was the true dysentery bacillus obtained from the same stools as these lactose fermenters.

In addition to the dysentery-like lactose fermenters, one often encounters, in cases of this character, bacilli which act on litmus milk in a way quite similar to B. dysenteriæ, with the exception of the fact that their final alkaline production is far more intense in degree than is the case with the dysentery bacillus, simulating in this respect the reaction of Bacillus alkaligenes (Petruschky). These forms have been encountered by Duval and Schorer,

Kendall, Wollstein, and others, and are designated by the first-named investigators as the "alkaline" bacillus. They grow on agar in a way which is entirely typical of the dysentery bacillus, but they are often slightly motile and many spread somewhat through the Hiss semi-solid medium. They never produce gas in any sugar medium, do not produce indol, nor do they agglutinate at all with the serum of rabbits immunized to any known strain of B. dysenteriæ. They do, however, reduce nitrates to nitrites. This bacillus, as Duval and Schorer conclude, is entirely outside the dysentery bacilli group. The many respects in which it resembles these organisms, especially the "Shiga-Kruse" type, make a careful study of its characters desirable. It is evidently identical with the form described by Ford under the appellation B. dysenteriæ (Müller).

BIOCHEMICAL CHARACTERS.

Fermentations.—From eight of the following cases organisms were isolated, which strongly resembled the dysentery bacillus in every particular except their ability to split lactose after a varying period of incubation. As a means of differentiation, Hiss' litmus-serum water, to which various sugars and mannit were added in the proportion of 1 %, was used.

The table given below is founded on a fourteen-day test.

All of these cultures act quickly on dextrose, mannit, and maltose, often reddening and coagulating the medium in twenty-four hours. In six of the cases, however, the organisms isolated do not ferment saccharose at all, not even after extended cultivation in association with this sugar. These organisms, which do not ferment saccharose, are further divided by the fact that some of them split dextrin while others do not. On this distinction Duval has called those which do not act on dextrin, Bacillus "A," and those which do, Bacillus "B." There are, however, many transition forms between these two groups and, as will be shown, they agglutinate alike and are practically identical. Duval and Flexner have pointed out that in regard

to their fermentative powers they resemble somewhat the "Y" and "Flexner-Harris" types.

If a dysentery-like culture splits maltose readily, but does not touch saccharose, it invariably, according to the experience of

TABLE IV.

Fermentations.—A Fourteen-Day Test.

Case Number.	Number of organisms tested from each case.	Dextrose.	Mannit.	Maltose.	Saccharose.	Lactose.	Dextrin.	Letter given to variety of Bacillus.
23	2	R & C	R & C	R & C	—	R	—	A
24	1	R & C	R & C	R & C	—	R	R & C	B
25	2 { 1	R & C	R & C	R & C	—	P	R & C	B
	2 { 1	R & C	R & C	R & C	—	—	—	A
26	1	R & C	R & C	R & C	—	—	—	A
27	2 { 1	R & C	R & C	R & C	—	—	—	A
	2 { 1	R & C	R & C	R & C	—	R & C	R & C	B
28	4 { 2	R & C	R & C	R & C	—	—	P	B
	4 { 2	R & C	R & C	R & C	—	P	P	B
29	4	R & C	R & C	R & C	R & C	R & C	R & C	C
30	4	R & C	R & C	R & C	R & C	R & C	R & C	C
31	2	R & C	—	—	R & C	—	—	D

R & C stands for reddening and coagulation of the medium.
R " " reddening of the medium.
P " " slight evidence of acidity.

the writer, belongs in this class of bacteria, and will be found to split lactose if kept under observation for three or four weeks. Six of the cultures described by Weaver and Tunnicliff as being of the "Flexner-Harris" type, evidently, from their table of fermentations, belong in this category.

There is great variation in the time required by these organ-

isms in the splitting of lactose. In some instances it took place as early as the ninth day, while in others it was delayed until the twenty-fifth day. As regards the time in which dextrin is attacked we find a like variability. In general, a culture which acts on dextrin quickly, also attacks lactose within two weeks. In two cases organisms were recovered which split all five sugars and the alcohol mannit with a reddening and coagulation of the medium. Dextrose, mannit, maltose, and saccharose media were reddened and coagulated within forty-eight hours, dextrin in four or five days, and finally lactose in six to thirteen days. To this form of bacillus the letter "C" is given. Although bearing a strong cultural resemblance, these organisms are entirely distinct from the type "A" and "B" and bear no agglutinative relationship either to them or to any type of Bacillus dysenteriæ.

Under Case 31 is described an organism, which is certainly identical with the "alkaline" dysentery-like bacillus described by Duval. For convenience' sake it has been given the letter "D" in this paper. It bears a superficial resemblance to the "Shiga-Kruse" type, but in addition to splitting dextrose it was found also to split saccharose in nine to twelve days with a reddening and coagulation of the medium. On no other sugar or mannit has it any effect.

Litmus Milk.—The changes which these lactose-splitting organisms produce in litmus milk are most interesting and important, inasmuch as a great deal of dependence has been placed on this medium as a means of identification of dysentery bacilli. When first isolated some of these organisms act on litmus milk in a manner so like that of the dysentery bacillus that it is only after an observation of two or three weeks that a difference can be detected. They all produce a slight primary and entirely typical acid reaction which changes after three or four days to amphoteric. According to Duval the change does not go farther, but it has been the experience of the writer that organisms identical with Bacillus "A" and "B" produce a distinct alkaline reaction. This may last for from six to twelve days. In two or three weeks, however, from the time the tubes were inoculated, a second production of acid sets in, which is much stronger than

the initial production. As is shown by the fermentation experiments, this second acidity is due to the breaking up of the lactose in the milk. Although the cultures were kept under observation for several weeks no coagulation of the medium occurred.

After the organisms have been under cultivation for some time, the duration of the alkaline period is considerably shortened, and in three or four weeks sufficient acid may be produced to coagulate the medium. There is, however, considerable variation in this respect. Some cultures do not seem to produce coagulation at all. These are the ones which either act lightly on dextrin or fail to split it at all. On the other hand, in two cultures which have been under cultivation for over a year, the whole series of changes, first initial acidity, then alkalinity, and finally strong acidity and coagulation, all take place within four days.

Turning now to the dysentery-like cultures which split saccharose and which have been designated as "C," we encounter a rather different form of reaction. When such organisms have recently been isolated, they may either react in the same manner as bacilli "A" and "B," or there may be no period of alkalinity between two periods of acidity. Even the former, under cultivation, tend to lose the stage of alkalinity, coagulation frequently taking place within a week.

It is interesting to note that we have a whole series of forms, some on the one hand nearly identical in their physiological activities with B. dysenteriæ, while others, at the opposite end of the series, simulate Bacillus coli, although there is no gas production and no motility in any instance, nor is there any agglutinative relationship to the colon group.

Bacillus "D" ("alkaline"), as has been noted by Duval and Schorer, gives rise to a series of changes in litmus milk, which are quite similar to those produced by the dysentery bacillus. The final alkaline reaction, however, is far more intense, and after a considerable period the medium may become jellified. Agglutination tests show that this bacillus is certainly entirely outside the dysentery group and in no way related to it. As has already

been indicated, in a number of features it resembles "B" alkaligenes (Petruschky), but it differs from that organism in its ability to split two sugars and in clouding the closed arm of the fermentation tube. Duval and Schorer encountered it very frequently, in at least three out of five cases. It has also been found by Wollstein and Kendall.

To summarize, then, we have the following four types of dysentery-like organisms, which have been recovered from the dejecta of cases of summer diarrhœa in infants.

Bacillus "A" (Duval and Schorer): attacks dextrose, mannit, maltose, and lactose; no action on dextrin or saccharose.

Bacillus "B" (Duval and Schorer): attacks dextrose, mannit, maltose, lactose, and dextrin; no action on saccharose.

Bacillus "C": attacks dextrose, mannit, maltose, lactose, dextrin, and saccharose.

Bacillus "D" ("alkaline" of Duval and Schorer): attacks dextrose and saccharose; causes a strong alkali production in litmus milk.

Agglutinations.—With normal rabbit serum Bacilli "A" and "B" of the lactose fermenters are often agglutinated in as high a dilution as 1:200 and in all cases up to 1:40. In this respect they resemble the culture "Baltimore" of the true dysentery bacilli group.

Two rabbits were immunized, one to a culture which did not attack dextrin or Bacillus "A," and the other to a culture which acted on dextrin within two weeks. The organisms were very slightly, if at all, pathogenic to rabbits. The technique described in the previous paper was followed. On account of the agglutinins present in the blood of normal rabbits, the lowest final dilution in each case was 1:100.

As will be observed in the following table, all the types of dysentery-like organisms were tested with the sera of rabbits immunized to seven types of dysentery or dysentery-like bacilli and also to typhoid, paratyphoid, and colon bacilli. The lactose-splitting, dysentery-like organisms always raised a large amount of agglutinin after a few inoculations.

The serum of a rabbit immunized to Group I ("Shiga-Kruse")

type of dysentery contained few agglutinins for Bacilli "A" and "B," hardly more than normal rabbit serum. Group III, Case 2, and Group IV, Case 4, raised a somewhat greater amount of agglutinin than Group I, but Group II "Y" and Group IV "Baltimore," the most of all. Agglutination with the last sera took place in dilutions up to one-tenth to one-fifth of their

TABLE V.

Agglutinations with Immune Sera.

Cultures.	Rabbit immunized to Bacillus A.	Rabbit immunized to Bacillus B.	Rabbit immunized to Group I (Dys.).	Rabbit immunized to Group II (Dys.).	Rabbit immunized to Group III (Dys.).	Rabbit immunized to ("Balt.") Group IV (Dys.).	Rabbit immunized to (Case 4) Group IV (Dys.).	Rabbit immunized to B. Typh.	Rabbit immunized to B. Paratyph.	Rabbit immunized to B. Coli.
Bacillus A, Case No. 23	Homologous 20,000	20,000	200	1,000	500	2,000	200	1,500	500	0
Bacillus B, Case No. 27	20,000	Homologous 20,000	200	1,000	200	1,000	200	1,500	0	0
Bacillus C, Case No. 29.	0	0	0	0	0	0	0	■	200	100
Bacillus D "alkaline.".	0	0	0	0	■	0	0	0	0	100
Group I "Kruse.".....	0	0	15,000							
Group II "Y".....	■	0		6,000						
Group III, Case No. 2.	500	0			8,000					
Group IV "Baltimore"	200	100				15,000				
Group IV, Case No. 4.	500	0					4,000			
Typhoid bacillus.....	200	0						40,000		
Paratyphoid bacillus..	■	0							40,000	
Colon bacillus.....	200	0								100,000

titer. In typhoid serum of a high value, "A" and "B" agglutinated as high as 1:1500. This is of considerable interest, as Duval has placed these organisms in an intermediate position between the dysentery and typhoid groups. The paratyphoid serum employed contained a small amount of agglutinin for these bacilli, but B. coli serum none at all. It will be observed that the cultures "A" and "B" agglutinate in practically an identical manner in all the sera.

Bacillus "C," although differing so slightly culturally from

"A" and "B," does not agglutinate at all in the serum of rabbits immunized to the latter, nor in fact to any dysentery, typhoid, or colon serum. The same is true of the "alkaline" Bacillus "D." This fact, of course, indicates that they bear no relationship to the dysentery group.

Turning now to the agglutinating power of the serum raised by "A" or "B," we find that it clumps the various types of dysentery bacilli either in very low dilutions or not at all. The same is true in regard to its action on typhoid, paratyphoid, and colon cultures. The serum raised by Bacillus "A," however, contains slightly more agglutinin for dysentery bacilli than does that raised by "B."

Absorptions.—The absorptions are interesting in that they show the following points:

(1) That Bacillus "A" and "B," although differing somewhat in their fermentative power, are practically identical in their agglutinative reactions.

BACILLUS "A" SERUM ABSORBED WITH BACILLUS "B".

Cultures.	After absorption.	Before absorption.
Bacillus "A"	0	20,000
" "B"	0	20,000
Group IV "Baltimore"	0	200

All of the major [1] and minor agglutinins are removed.

(2) That a dysentery-like organism splitting lactose absorbs none of the major or minor agglutinins from a dysentery bacillus serum.

GROUP IV, "BALTIMORE" SERUM ABSORBED WITH BACILLUS "A."

Cultures.	After absorption.	Before absorption.
Bacillus "A"	0	2,000
Group II "Y"	1,000	1,000
" III, Case 2	1,000	1,000
" IV "Baltimore"	15,000	15,000

[1] Major agglutinin is used here as synonymous with the term "chief agglutinin," and minor with the term "common agglutinin."

GROUP II "Y" SERUM ABSORBED WITH BACILLUS "A"

Cultures.	After absorption.	Before absorption.
Bacillus "A"	0	1,000
Group II, Case 16	12,000	12,000
" IV "Baltimore"	2,000	2,000
Typhoid	200	200

Neither the major nor minor agglutinins for dysentery or typhoid bacilli are affected.

(3) That a dysentery culture absorbs none of the major agglutinins from the serum of a rabbit immunized to a dysentery-like lactose-splitting organism, but that it does absorb all minor agglutinins for all types of dysentery bacilli.

BACILLUS "A" SERUM ABSORBED WITH GROUP IV "BALTIMORE."

Cultures.	After absorption.	Before absorption.
Bacillus "A"	20,000	20,000
Group II, Case 3	0	500
" III, " 2	0	1,000
" IV "Baltimore"	0	2,000

BACILLUS "A" SERUM ABSORBED WITH GROUP III, CASE 5.

Cultures.	After absorption.	Before absorption.
Bacillus "A"	20,000	20,000
Group III, Case 5	0	500
" IV "Baltimore"	0	2,000

None of the major agglutinins are removed, but all the minor for several types of dysentery bacilli. The last absorption also seems to show that the culture "Baltimore" requires less agglutinating substance to bring about its agglutination than does culture of Group III., Case 5.

(4) Finally, that none of the major agglutinins are absorbed

from typhoid serum by either Bacillus "A" or "B," and that the minor agglutinins in this serum for dysentery bacilli are partially absorbed by "A," but not at all by "B."

TYPHOID SERUM ABSORBED WITH BACILLUS "A."

Cultures.	After absorption.	Before absorption.
Bacillus "A"	0	1,000
Typhoid	40,000	40,000
Group II, Case 16	200	500
" IV "Baltimore"	200	1,000

TYPHOID SERUM ABSORBED WITH BACILLUS "B."

Cultures.	After absorption.	Before absorption.
Bacillus "B"	0	1,000
Typhoid	20,000	20,000
Group II "Y"	200	200
" IV "Baltimore"	1,000	1,000

From the above absorptions it seems evident that Bacillus "A" bears a slightly closer relationship to dysentery bacilli than Bacillus "B."

In both of the rabbits immunized to these dysentery-like organisms, a very decided proagglutinoid zone appeared after a considerable number of inoculations had been given. In the case of the serum from the rabbit immunized to Bacillus "A" the zone was highest after ten agar cultures had been given extending over a period of about four months. At this time it was very marked in dilutions of 1: 200. The serum from the rabbit immunized to Bacillus "B" also showed a proagglutinoid zone in a little over two months and after eight cultures had been given. It had entirely disappeared, however, in five months and after the inoculation of fifteen cultures.

GENERAL REMARKS.

In placing these dysentery-like bacilli, which split lactose with a greater or less degree of celerity, within the group of pseudo-

dysentery organisms, it is well to bear in mind that the term "pseudo-dysentery" has at present a very indefinite significance. Kruse was the first to use it in connection with his studies of the bacteriology of dysentery of the insane. The organisms which he isolated were, according to his tests, similar culturally to his dysentery bacillus, but differed from it in their agglutination reactions. These cultures from the insane were, however, as has been emphasized by Hiss, in all probability identical with the mannit-splitting culture known as Flexner (Grey or Harris). Lentz also placed Flexner's cultures among the pseudo-dysentery bacilli. It is obvious, nevertheless, from our present knowledge, that these two principal types of dysentery bacilli—the "Shiga-Kruse" and the "Flexner-Harris"—are of co-equal importance, and that neither can justly be called a pseudo-dysentery form. Most of the other bacilli classed by Martini and Lentz are either gas producers or actively motile. Ford, on the other hand, has given the name Bacillus pseudo-dysenteriæ (Müller) to organisms which are evidently identical with the "alkaline" Bacillus "D" and which bear a superficial resemblance to the "Shiga-Kruse" type. Although he names Müller as the one who gave the original description to such forms, there seems to be no evidence in the article to which he refers that such is the case. The only cultures which Müller describes in his article are seven, which are manifestly true dysentery organisms of the "Shiga-Kruse" type, and two which are non-motile and non-gas-producing, but which coagulate milk in twenty-four hours. As the author remarks, these last are very similar to the Bacillus coli anaërogenes (Lembke), with the exception of the fact that they produce no change in neutral red. It would seem to be more reasonable to affix the name of Kruse or Lentz as the originators of the group, for although they included certain true dysentery organisms in this group, they were the first to describe other dysentery-like organisms to which this name might well be attached.

It is questionable, nevertheless, whether any organisms similar to Bacilli "A" and "B" were noted by these investigators. One culture described by Martini and Lentz split dextrin, dex-

trose, and mannit without gas production, but had no effect on saccharose. It agglutinated with patients' blood in dilutions of 1:50. Whether or not it split lactose eventually was not stated, but, as has already been pointed out, a dysentery-like organism which acts on dextrin, dextrose, and mannit, but not on saccharose, is almost certainly identical with Bacillus "B."

During the investigations of the diarrhœal diseases of infancy carried out by the Rockefeller Institute, organisms very similar to dysentery bacilli, which produce a second and stronger acid reaction in litmus milk, were encountered, not only by Duval and Schorer, but also by Kendall and Gay. Weaver and Tunnicliff, in addition, have noted the occurrence of organisms which were dysentery-like, but which failed to give typical agglutination reactions. Under the title of "Another Member of the Dysentery Group," Duval has recently described in detail an organism identical culturally with his Bacillus "B," which seemed to occupy, as regards the agglutinins present in the patient's blood and its clumping in various immune sera, an intermediate position between the typhoid bacillus and the dysentery bacilli. This bacillus was recovered from a fatal case of dysentery in an adult. As has already been shown, the cultures of this bacillus, which were obtained by the writer, agglutinate to a certain extent in typhoid and dysentery immune sera, but in much lower dilutions, proportionally, than has been described by Duval. The same is true also in regard to the limit of agglutination of typhoid and dysentery bacilli in the serum of a rabbit immunized to Bacillus "A" or "B." The fact that all the agglutination tests described in this article were made macroscopically may account for the difference in results.

These cultures, in fact, seem to give evidence of only a slight relationship to the typhoid bacillus. It is possible that they may be aberrant derivatives of the dysentery group. Their ability to split lactose, certainly, seems to indicate that they have become well adapted to a saprophytic existence. The extent of this adaptation may well be measured by the rapidity with which this sugar is split. Bacillus "C" has all the characteristics of a saprophyte and is farthest removed from the true dysentery

organism. On the other hand, the tendency of evolution might be, and is, perhaps, better considered to be, in the other direction, i. e., the true dysentery organism has sprung from some such lactose fermenter, and by a gradual change of habitat and adaptation to a purely parasitic existence has lost its power to act on one sugar after another until we find the "Shiga-Kruse" type, which splits only dextrose and which is certainly most pathogenic in its properties.

The more closely that this whole dysentery group is studied the greater seems to be the difficulty in defining its limits and in determining what forms shall be and what shall not be called dysentery bacilli. It is quite possible that these dysentery-like organisms, which split lactose, may be an etiological factor in some of the milder diarrhœas of infants, and also may at times assume a very pathogenic and possibly invasive character, as evidenced by the case reported by Duval. That they should be admitted to the dysentery group, however, seems hardly justified by what we know at present of their occurrence and properties. An extended series of agglutination experiments with the blood of the individuals from whom they were isolated would certainly decide this question definitely. Possibly the best plan, at present, is to include in the pseudo-dysentery group all bacilli of a typical dysentery morphology, which give rise to a primary acid reaction in litmus milk followed by a return to amphoteric, which are not motile, which do not liquefy any media, and which do not produce gas. Essentially the same suggestion has already been made by Ford. This group might well be divided as follows:

Division I. Organisms, corresponding to the description given above, which act slowly on lactose and do not split saccharose, but which agglutinate in the serum of animals immunized to dysentery cultures splitting mannit. Examples: Bacillus "A" and Bacillus "B."

Division II. Organisms, corresponding to the description given above, which do not agglutinate in any anti-dysenteric serum. Examples: Bacillus "C" and Bacillus "D."

It is evident that these pseudo-dysentery organisms may be

and perhaps have been in some instances mistaken for true Bacillus dysenteriæ. Investigators should bear in mind that they are of common occurrence. Especially are they liable to be confused with the "Y" and "Flexner-Harris" types. The most reliable differential test between the pseudo and the true dysentery bacilli is litmus milk, the cultivation being continued over a period of two or three weeks. The agglutination test is also an aid in detecting these pseudo forms, but it should be borne in mind that some of them clump in quite high dilutions in the serum of animals immunized to mannit-splitting dysentery bacilli.

A general summary of the characters in which these pseudo forms, which split lactose, resemble and differ from true B. dysenteriæ may be made as follows.

I. Characters in which they are identical.

In morphology, in lack of motility, in not producing spores, in producing little or no pellicle on broth, in not clouding Hiss semi-solid medium, in not producing gas, in not liquefying any media, in producing a primary acidity in litmus milk followed by an alkaline reaction, and in the fact that pseudo-dysentery bacilli belonging in Division I agglutinate in the serum of rabbits immunized to mannit-splitting dysentery bacilli.

II. Characters by which the lactose-fermenting organisms are separated from the dysentery bacillus.

In a greater spreading on the surface of agar and gelatine, in producing a final acid reaction in litmus milk, in not absorbing any of the major or minor agglutinins for dysentery bacilli from the serum of animals immunized to any of the various groups of the dysentery bacillus, in not being at all pathogenic to rabbits, in failing to agglutinate according to the experience of the writer in the blood of the individuals from whom they were isolated in the dilutions ordinarily employed.

The points of resemblance indicate a general or family relationship between the pseudo and the true dysentery bacillus groups. This relationship may be, perhaps, well compared to that existing between the true and the pseudo-diphtheria bacillus groups. On the other hand, the characters in which these two groups differ,

especially the lack of common agglutinins, indicate that they must be regarded as distinct species. The pseudo forms, through their power of splitting lactose, are well adapted to a saprophytic existence and apparently are devoid of active pathogenic properties.

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